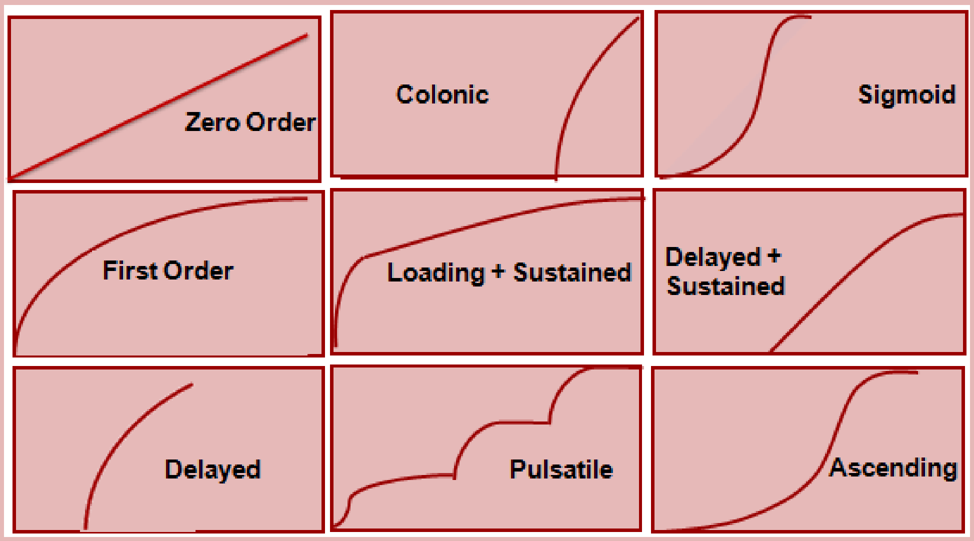
Drug Delivery Systems:
A Review



Editor

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Dedicated to pharmacy profession

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PREFACE

Men and medicine are inseparable from times immemorial. Although the physical forms of medication have not changed dramatically, the attitude of the public toward accepting medicines have changed with the passage of time.

This fact is also reflected in the strategies adopted by the pharmaceutical companies in the field of research. The cost involved, both in terms of time and money, has made it mandatory for the companies to reconsider their research focus. In an attempt to reduce the cost of drug development process and advantageously reap the benefits of the patent regime, drug delivery systems have become an integral part of the said process.

Drug delivery system is a dosage form, containing an element that exhibits temporal and/or spatial control over the drug release. The ultimate aim of such systems is tailoring of the drug formulation to individual requirements under the control of pathophysiological or in-vivo conditions rather than in-vitro characteristics.

This field of drug delivery systems is dynamic and extensive. Probably it would need an encyclopedia to cover all the types of drug delivery systems. The aim of this book is to compile major drug delivery systems and offer a source of information for all those working in pharmaceutical academia as well as industry.

The book is made available free of charge to all who are interested in the subject for dissemination of knowledge. Authors feel proud to be a part of first of its kind of experiment wherein a technical book is offered for free download through a blog.

We welcome suggestions and criticisms for our readers.

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Table of contents

1. Fundamentals of Drug Delivery System - 10
   Suryavanshi Kiran, Mogal Rajendra, Pawar Yogesh, Shaikh Aamir

2. Oral Controlled Drug Delivery System - 18
   Bajaj Amruta, Katedeshmukh Ramesh

3. Gastroretentive Drug Delivery System - 43
   Basrur Pooja, Hastak Vishakha

4. Colon Specific Drug Delivery System - 59
   Bhuruk Manisha, Pawar Yogesh

5. Chronopharmaceutical Drug Delivery System - 83
   Chavan Shankar, Shaikh Aamir

6. Self Dispersing Formulations-101
   Deshpande Tanvee, Mogal Rajendra

7. Introduction To Bioadhesion/Mucoadhesion - 114
   Kamble Pranay, Katedeshmukh Ramesh

8. Mucoadhesive Drug Delivery System - Nasal - 127
   Khan Halimunnisa, Hastak Vishakha

9. Mucoadhesive Drug Delivery System - Rectal - 149
   Kulkarni Akshasa, Pawar Yogesh

10. Mucoadhesive Drug Delivery System - Vaginal - 160
    Deshpande Tanvee, Shinde Rohit, Pawar Yogesh

11. Parenteral Controlled Drug Delivery System – 182
    Maravaniya Pathikkumar, Shaikh Aamir

12. Parenteral Implants – 194

Patel Ruchita, Mogal Rajendra

13. Transdermal Drug Delivery System - 206

Pawar Sandesh, Katedeshmukh Ramesh

14. Particulate Drug Delivery System-Liposomes - 224

Satam Madhavi, Hastak Vishakha

15. Particulate Drug Delivery System- Microcapsules– 241

Sawant Sandip, Pawar Yogesh

16. Particulate Drug Delivery System- Microspheres -253

Sawant Sandip, Pawar Yogesh

17. Particulate Drug Delivery System-Resealed Erythrocytes-266

Shinde Rohit, Shaikh Aamir

18. Particulate Drug Delivery System-Monoclonal Antibodies -281

Suryavanshi Kiran, Mogal Rajendra

19. Intranasal Drug Delivery System - 291

Wayal Abhijit, Katedeshmukh Ramesh

20. Protein And Peptide Drug Delivery System - 302

Zarikar Nitin, Hastak Vishakha

21. Intraocular Drug Delivery System - 318

Maravaniya Pathikkumar , Zarikar Nitin , Pawar Yogesh

22. Pulmonary Drug Delivery System – 326

Kamble Pranay, Suryavanshi Kiran, Shaikh Aamir

23. Nanopharmaceuticals – 334

Kulkarni Akshada, Patel Ruchita, Mogal Rajendra

24. Medicated Chewing Gums – 347
Basrur Pooja, Katedeshmukh Ramesh

25. Oral Thin Film – 357
Bhuruk Manisha, Satam Madhavi, Hastak Vishakha

26. Nail Drug Delivery System – 367
Basrur Pooja, Suryavanshi Kiran, Katedeshmukh Ramesh

27. Regulatory Aspects of Drug Delivery System- 377
Chavan Shankar, Mogal Rajendra, Pawar Yogesh, Shaikh Aamir

FUNDAMENTALS OF DRUG DELIVERY SYSTEMS

Suryavanshi Kiran, Mogal Rajendra, Pawar Yogesh, Shaikh Aamir

Need for Controlled Release Systems:

(Kathryn E. Uhrich 1999)Controlled drug delivery technology represents one of the most rapidly advancing areas of science in which chemists and chemical engineers are contributing to human health care. Such delivery systems offer numerous advantages compared to conventional dosage forms including improved efficacy, reduced toxicity, and improved patient compliance and convenience. Such systems often use synthetic polymers as carriers for the drugs. By so doing, treatments that would not otherwise be possible are now in conventional use. Although the introduction of the first clinical controlled release systems occurred less than 25 years ago, 1997 sales of advanced drug delivery systems in the United States alone were approximately $14 billion dollars. Synthetic polymers used in the controlled release of drugs. Before considering the variety and the evolution of these polymeric structures, it is necessary to examine the motivation for achieving controlled release. This field of pharmaceutical technology has grown and diversified rapidly in recent years. Understanding the derivation of the methods of controlled release and the range of new polymers can be a barrier to involvement from the nonspecialist. All controlled release systems aim to improve the effectiveness of drug therapy. This improvement can take the form of increasing therapeutic activity compared to the intensity of side effects, reducing the number of drug administrations required during treatment, or eliminating the need for specialized drug administration (e.g., repeated injections).

B. Methods of Controlled Release

In temporal control, drug delivery systems aim to deliver the drug over an extended duration or at as specific time during treatment.

Controlled release over an extended duration is highly beneficial for drugs that are rapidly metabolized and eliminated from the body after administration. An example of this benefit is shown schematically in Figure 1 in which the concentration of drug at the site of activity within the body is compared after immediate release from 4 injections administered at 6 hourly intervals and after extended release from a controlled release system. Drug concentrations may fluctuate widely during the 24 h period when the drug is administered via bolus injection, and for only a portion of the treatment period is the drug concentration in the therapeutic window (i.e., the drug concentration that produces beneficial effects without harmful side effects). With the controlled release system, the rate of drug release matches the rate of drug elimination and, therefore, the drug concentration is within the therapeutic window for the vast majority of the 24 h period. Clinically, temporal control can produce a significant improvement in drug therapy. For example, when an opioids pain killer is administered to a patient with terminal cancer, any time that the drug concentration is below therapeutic concentrations the patient experiences pain. A temporally controlled release system would ensure that the maximum possible benefit is derived from the drug. In distribution control, drug delivery systems aim to target the release of the drug to the precise site of activity within the body. The benefit of this type of control is shown schematically in Figure 2 in which

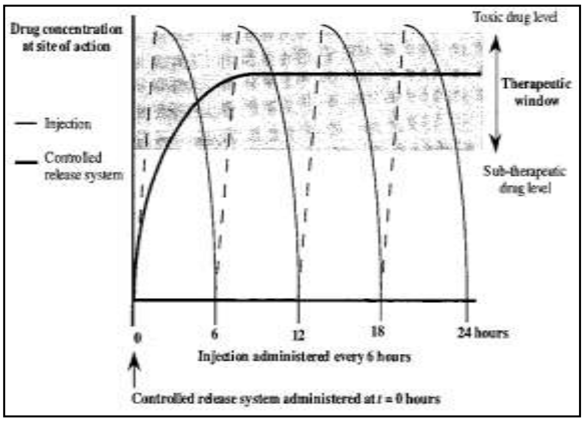

Figure 1. Drug concentrations at site of therapeutic action after delivery as a conventional injection (thin line) and as a temporal controlled release system (bold line). (Kathryn E. Uhrich 1999)

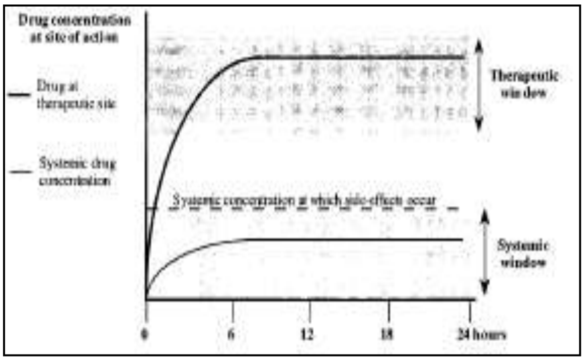

Figure 2. Drug delivery from an ideal distribution controlled release system. Bold line: Drug concentrations at site of therapeutic action. Thin line: Systemic levels at which side effects occur. (Kathryn E. Uhrich 1999)

Drug concentrations at the site of activity and side effect production are compared. There are two principle situations in which distribution control can be beneficial. The first is when the natural distribution causes drug molecules to encounter tissues and cause major side effects that prohibit further treatment. This situation is often the cause of chemotherapy failure when bone marrow cell death prevents the patient from undergoing a complete drug treatment. The second situation is when the natural distribution of the drug does not allow drug molecules to reach their molecular site of action. For example, a drug molecule that acts on a receptor in the brain will not be active if it is distributed by the patient's blood system but cannot cross the blood-brain barrier. A large number of classes of drugs can benefit from temporal or distribution controlled release. These classes include chemotherapeutic drugs, immunosuppressants, anti-inflammatory agents, Antibiotics, opioid antagonists, steroids, hormones, anesthetics, and vaccines. Recently, the need to develop new controlled release strategies has been intensified by advances in the design of peptide drugs and emergence of gene therapy. These biotechnology derived agents may dominate the next generation of drug design. However, their clinical success may be dependent on the design of controlled release devices that ensure that the drugs reach their target cells precisely at the required time. A discussion of the pharmacological and clinical motivations for controlling the release of the specific drug classes referred to above is beyond the limit of this article; however, a number of excellent reviews are available. In addition, it should be noted that controlled release technology is not confined to pharmaceutical applications but has also proven beneficial in agricultural and cosmetic industries. (Kathryn E. Uhrich 1999)

Scope of Polymer Systems:

In this review, a number of polymer backbones that are potentially degradable are detailed in the text. This restriction certainly does not reduce the impact and significance of C-C backbones for controlled release applications but is simply a mechanism to focus on an important subset of materials. To illustrate the diverse range of functionalities available from nonbiodegradable systems based on C-C backbones to heteroatom-containing polymer backbones that may confer biodegradability. (Langer 1998)

Mechanisms of Controlled Drug Release Using Polymers:

A diverse range of mechanisms have been developed to achieve both temporal and distribution controlled release of drugs using polymers. This diversity is a necessary consequence of different drugs imposing various restrictions on the type of delivery system employed. For example, a drug that is to be released over an extended period in a patient's stomach where the pH is acidic and environmental conditions fluctuate widely will require a controlled release system very different from that of a drug that is to be delivered in a pulsatile manner within the blood system. An important consideration in designing polymers for any controlled release mechanism is the fate of the polymer after drug release. Polymers that are naturally excreted from the body are desirable for many controlled release applications. These polymers may be excreted directly via the kidneys or may be biodegraded into smaller molecules that are then excreted. Nondegradable polymers are acceptable in applications in which the delivery system can be recovered after drug release (e.g., removal of patch or insert) or for oral applications in which the polymer passes through the gastrointestinal tract. From a polymer chemistry perspective, it is important to appreciate that different mechanisms of controlled release require polymers with a variety of physicochemical properties. This requirement has stimulated the evolution of the new polymers that will be discussed in section IV. Before consideration of these polymers, the major mechanisms of controlled release and polymeric characteristics that are required to carry out these mechanisms will be briefly. (Kathryn E. Uhrich 1999)

CLASSIFICATION OF DRUG DELIVERY SYSTEM:

Classification of NDDS based on Physical means

1) Osmotic Pressure Activated
2) Hydrodynamic pressure activated
3) Vapor pressure activated
4) Mechanically activated
5) Magnetically activated
6) Sonophoresis
7) Iontophoresis
8) Hydration activated

Classification of NDDS based on Chemical means

1) Hydrolysis activated
2) Ion activated
3) pH activated

Polymers Generally Used for Controlled Drug Delivery System:

1) **Poly(esters):**

Poly (esters) is the best characterized and most widely studied biodegradable system. The synthesis of poly (esters) has received as much attention as the degradation of these materials. A patent for the use of poly (lactic acid) (PLA) as a resorbable suture material was first filed in 1967.34 The mechanism of degradation in poly (ester) materials is classified as bulk degradation with random hydrolytic scission of the polymer backbone

Polymerization of the cyclic lactone alone is usually too slow to produce high molecular weight material (>20 000 amu). The rate of ring opening for the cyclic lactone can be increased by activation of a Zn- or Snbased catalyst with the carbonyl ester. However, the introduction of a catalyst invites concerns over traces of potentially cytotoxic material. Thus, stannous octoate SnII (CO2CH(*n*Bu)(Et))2 is commonly used because has FDA approval as a food stabilizer.

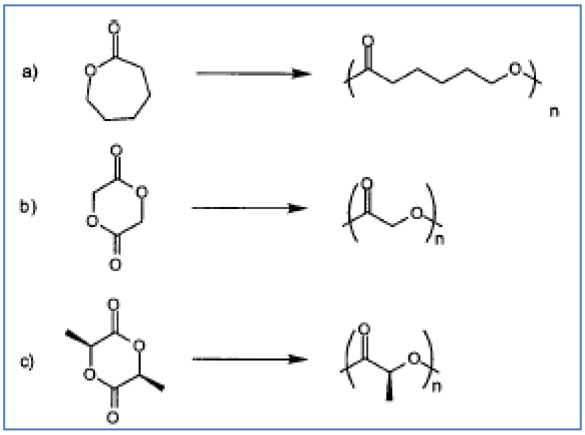

Fig:3 Ring- opening polymerization of selected cyclic lactones to give the following

A) Poly(e-caprolactone)PCL
B) Poly(glycolic acid) PGA
C) Poly(L-lactic acid)PLA (Kathryn E. Uhrich 1999)

1. **Poly(lactic acid), Poly(glycolic acid**), and Their Copolymers Poly(esters) based on poly(lactic acid) (PLA), poly- (glycolic acid) (PGA), and their copolymers, poly(lactic acid-*co*-glycolic acid) (PLGA), are some of the best defined biomaterials with regard to design and performance. Lactic acid contains an asymmetric R-carbon which is typically described as the D or L form in classical stereo chemical terms and sometimes as the *R* and *S* form, respectively. For homopolymers, the enantiomer forms are poly (D-lactic acid) (PDLA) and poly (L-lactic acid) (PLLA). The physicochemical properties of optically active PDLA and PLLA are nearly the same, whereas the racemic PLA has very different characteristics.41 For example, racemic PLA and PLLA have *T*g's of 57 and 56 °C, respectively, but PLLA is highly crystalline with a *T*m of 170 °C and racemic PLA is completely amorphous.

Because the naturally occurring lactic acid is L (or *S*), PLLA is considered more biocompatible. The polymers are derived from monomers that are natural metabolites of the body; thus degradation of these materials yields the corresponding hydroxy acid, making them safe for in vivo use. Biocompatibility of the monomer is the foundation for biocompatibility of degradable polymer systems. To this end, the degradation products often define the biocompatibility of a polymers not necessarily the polymer itself. Even though PLGA is extensively used and represents the gold standard of degradable polymers, increased local acidity due to the degradation can lead to irritation at the site of the polymer employment. Introduction of basic salts has been investigated as a technique to control the pH in local environment of PLGA implants

From a physical level of understanding, poly (esters) undergo bulk degradation. PLA homopolymers degrade slower than PGA homopolymers on the basis of crystallinity as well as stearic inhibition by the pendent methyl group of PLA to hydrolytic attack. However, the complexity of PLA, PGA, and PLGA degradation has been demonstrated by Vert45 and does not conform to a simple model. Vert and coworkers have demonstrated that size dependence for hydrolytic degradation exists for PLA systems. Other research efforts suggest that PLA-derived micro particles will degrade faster than nanoparticles derived from PLA. This is modeled on diffusion reaction phenomena. An autocatalytic effect at the interior of larger devices is thought to contribute to the initial heterogeneous degradation of larger devices as acidic byproducts cannot readily diffuse out from the interior as is the case for smaller constructs. Extensive degradation studies have also been reported for PLA, poly (caprolactone) (PCL), and their copolymers both in vitro and in vivo.

Studies in hydrolytic degradation for poly (esters) have focused on understanding the effects of changes in polymer chain composition. A distinguishable effect based on end group composition for poly (ester) degradation demonstrated that terminal carboxyl groups have a catalytic effect on hydrolysis for PGA. The ability to tailor rates of protein release from PLGA microspheres was derived from the understanding of end-group effects. The commercial developmental process for formulating poly (esters) with selected drug candidates has been reviewed. The aforementioned review highlights the development of poly (ester) matrices containing human growth hormone that sustained levels of a therapeutic protein in humans for 1 month from a single dose. (Kathryn E. Uhrich 1999)

2. **Poly (ethylene glycol) Block Copolymers:**

Poly (ethylene glycol) (PEG) is also referred to as poly (ethylene oxide) (PEO) at high molecular weights. Biocompatibility is one of the most noted advantages of this material. Typically, PEG with molecular weights of 4000 amu is 98% excreted in man. One of the emerging uses for inclusion of PEG in a controlled release system arises from its protein resistivity. The hydrophilic nature of PEG is such that water hydrogen bonds tightly with the polymer chain and thus excludes, or inhibits, protein adsorption. Many research groups are investigating attachment of PEG chains to therapeutic proteins; PEG chains at the surface allow for longer circulation of the protein in the body by prolonging biological events such as endocytosis, phagocytosis, liver uptake and clearance, and other adsorptive processes.

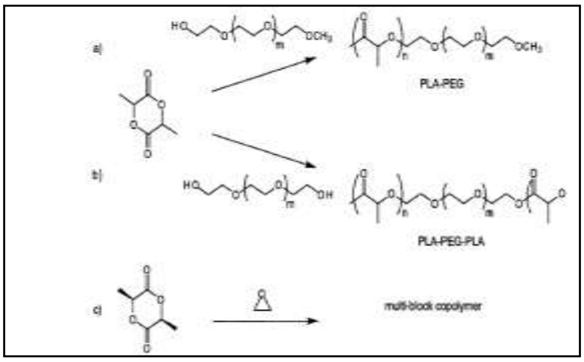

Fig.4 Synthesis of PLA-PEG Copolymer (Kathryn E. Uhrich 1999)

PEG can be made with a range of terminal functionalities which lends to its easy incorporation into copolymer systems. PEG is commonly terminated with chain-end hydroxyl groups which provide a ready handle for synthetic modification. Diblock PLA/ PEG and triblock PLA/PEG/PLA systems have been synthesized and characterized with various PLA contents. The free hydroxyl groups of PEG are ring-opening initiators for lactide in forming the diblock or triblock materials (Figure 5a, b). Recently, Chen et al. have synthesized PLA-PEG multiblock copolymers from L-lactide and ethylene oxide, the monomer precursors for PLA and PEG, respectively (Figure 5c). This approach is different in two respects: (i) use of bimetallic catalysts which proceed by anionic mechanisms; (ii) multiblock polymers are generated. Han and Hubbell further demonstrated the synthetic utility for PLA-PEG systems by introducing acrylate moieties to form cross-linked systems. Similarly, Jeong et al. prepared thermo sensitive PLA-PEO hydrogels that exhibit temperature-dependent gel-sol transition for use as injectable drug delivery systems.

Poly (ortho esters):

The motivation for designing poly (ortho esters) for drug delivery was the need to develop biodegradable polymers that inhibited drug release by diffusion mechanisms and allowed drug release only after the hydrolysis of polymer chains at the surface of the device.70 Most research on poly (ortho esters) has focused on the synthesis of polymers by the addition of polyols to diketene acetals. For example, Heller et al. have described the synthesis and application of the 3, 9-diethylidene-2, 4, 8, 10-tetraoxaspiro [5.5] undecane (DETOSU)-based poly (ortho esters).71 The basic structure is formed by the addition of the DETOSUmonomer to a diol to form the chemical structure. The DETOSU-based poly (ortho esters) contain acid labile ortho ester linkages in their backbone structure. Within aqueous environments, the ortho ester groups are hydrolyzed to form Pentaerythritol dipropionate and diol monomers as breakdown products. The Pentaerythritol dipropionate is further hydrolyzed to Pentaerythritol and acetic acid. Acid-catalyzed hydrolysis of these polymers can be controlled by introducing acidic or basic Excipients into matrixes. Rates of hydrolysis can be increased by the addition of acidic excipients, such as suberic acid, as demonstrated by the zero-order release of 5-fluorouracil over a 15 day period.72 Alternatively, basic excipients stabilize the bulk of the matrix but diffuse out of the surface region, thereby facilitating surface-only erosion. This approach has been employed in the temporal controlled release of tetracycline over a period of weeks in the treatment of periodontal disease.

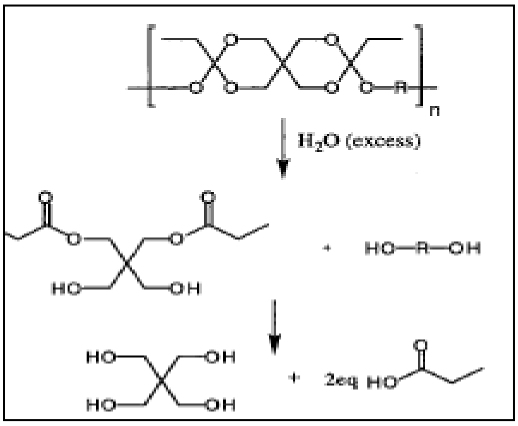

Fig.5: Degradation of 3, 9(bis ethylidene-2, 4,8,10 Tetraoxaspiro undecane (DETOSU) based poly ortho ester (Kathryn E. Uhrich 1999)

A useful feature of the DETOSU systems is the ability to control the mechanical properties by changing the diol monomer ratios within the final polymeric structure. For example, Heller et al. have shown that the glass transition temperature of polymers containing a rigid diol monomer (*trans*cyclohexanedimethanol) and a flexible monomer (1, 6- hexanediol) could be varied between 20 and 105° by increasing the proportion of the rigid diol. This control can also be achieved with the glycolide containing polymers.

A number of applications have been described for cross-linked poly (ortho esters) formed by the substitution of 1, 2, 6-hexanetriol for 1, 2-hexanediol, for example. The triol monomer allows cross-linked materials to be formed that are semisolid materials. It has been envisaged that these materials could be injected into the patient as a viscous liquid at slightly elevated temperatures that form nondeformable depot implants upon cooling. (V. Balmurlidhara 2011)

Poly (anhydrides)

To obtain a device that erodes heterogeneously, the polymer should be hydrophobic yet contain water sensitive linkages. One type of polymer system that meets this requirement is the poly (anhydrides). Poly-(anhydrides) undergoes hydrolytic bond cleavage to form water-soluble degradation products that can dissolve in an aqueous environment, thus resulting in polymer erosion. Poly (anhydrides) are believed to undergo predominantly surface erosion due to the high water liability of the anhydride bonds on the surface and the hydrophobicity which prevents water penetration into the bulk. This process is similar to the slow disappearance of a bar of soap over time. The decrease in the device thickness throughout the erosion process, maintenance of the structural integrity, and the nearly zero-order degradation kinetics suggest that heterogeneous surface erosion predominates. The majority of poly (anhydrides) are prepared by melt-condensation polymerization. Starting with a dicarboxylic acid monomer, a prepolymer of a mixed anhydride is formed with acetic anhydride. The final polymer is obtained by heating the prepolymer under vacuum to remove the acetic anhydride byproduct. The most widely studied poly (anhydrides) are based on sebacic acid (SA), *p*-(carboxyphenoxy) propane (CPP), and *p*-(carboxyphenoxy) hexane (CPH)

Degradation rates of these polymers can be controlled by variations in polymer composition. The more hydrophobic the monomer, the more stable the anhydride bond is to hydrolysis. Aliphatic poly- (anhydrides) (e.g., SA) degrade within days whereas aromatic poly (anhydrides) (e.g., CPH) degrade over several years.

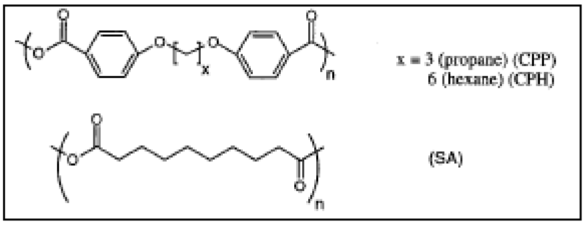

Fig: 6 Structure of widely used aromatic poly (anhydrides) based on monomer of p-carboxy phenoxy propane (Kathryn E. Uhrich 1999)

The biocompatibility of copolymers of SA and CPP has been well established. Evaluation of the toxicity of poly (anhydrides) show that they possess excellent in vivo biocompatibility.81 Recent clinical trials have demonstrated that an intracranial device of SA/CPP copolymers improves the therapeutic efficacy of an antitumor agent, bischloronitrosourea, for patients suffering from a lethal type of brain cancer.

Poly (anhydride-esters)

Other modifications of poly (anhydrides) include poly (anhydride-esters), which include two different types of hydrolytically cleavable bonds in the polymer backbone. In one example, low molecular weight carboxylic acid-terminated prepolymer of poly (_- caprolactone) were coupled via anhydride linkages. The intent of this research was to design polymers that displayed two-stage degradation profiles: anhydride bonds rapidly hydrolyzed to the poly (ester) prepolymer which degraded much more slowly. In another example, carboxylic acid-terminated monomers that contain ester bonds are activated and then polymerized using the same chemistry described for the poly (anhydrides). A unique aspect of these poly (anhydride-esters) is that hydrolytic degradation of the polymer backbone yields a therapeutically useful compound, salicylic acid. Polymer’s degradation products are potentially beneficial

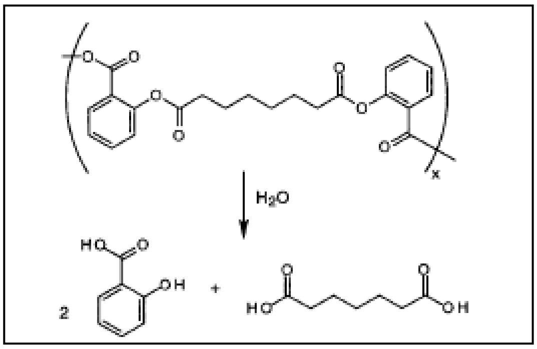

Fig: 7 Poly (anhydride ester that undergo into salicylic acid, an anti inflammatory agent (Kathryn E. Uhrich 1999)

Poly (amides):

The most interesting class of poly (amides) for controlled release are the poly (amino acids). The synthetic ability to manipulate amino acid sequences has seen its maturity over the last two decades with new techniques and strategies continually being introduced. An excellent review of the histo4ry of amino acid-derived polymers is given by Nathan and Kohn.93 Poly(amino acids) have been used predominantly to deliver low molecular weight drugs, are usually tolerated when implanted in animals,94 and are metabolized to relatively nontoxic products. These results suggest good biocompatibility, but their mildly antigenic nature makes their widespread use uncertain. Another concern with poly (amino acids) is the intrinsic hydrolytic stability of the amide bond which must rely upon enzymes for bond cleavage. The dependence on enzymes generally results in poor controlled release in vivo. The expense and difficulty in production of elaborate polypeptide sequences has limited the composition to homopolymers, predominantly poly (glutamic acid) and poly (aspartic acid). Poly(amino acids) are generally hydrophilic with degradation rates dependent upon hydrophilicity of the amino acids.96,97 Amino acids are attractive due to the functionality they can provide a polymer. For example, poly (lactic acid-*co* lysine) (PLAL) was synthesized using a stannous octoate catalyst from lactide and a lysine-containing monomer analogous to lactide. Inclusion of the amino acid lysine provides an amino group that allows for further modification of the PLAL system. Recently, peptide sequences that promote cell adhesion have been attached to PLAL.

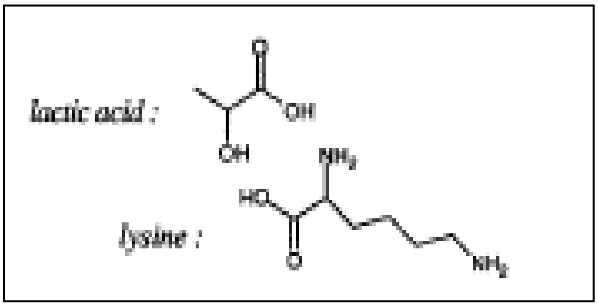

Fig.8 Poly (lactic acid-co-amino acid) PLAL Polymer system (Kathryn E. Uhrich 1999)

Currently marketed oral controlled-release systems:

Advances in oral controlled-release technology are attributed to the development of novel biocompatible polymers and machineries that allow preparation of novel design dosage forms in a reproducible manner. The main oral drug-delivery approaches that have survived through the ages are as follows:

- Coating technology using various polymers for coating tablets, nonpareil sugar beads, and granules
- Matrix systems made of swell able or nonswellable polymers
- Slowly eroding devices
- Osmotically controlled devices.

Conventional tablet formulations are still popular in the design of single-unit, matrix-type controlled release dosage forms. The advancement of granulation technology and the array of polymers available with various physicochemical properties (such as modified cellulose or starch derivatives) have made the development of novel oral controlled release systems possible. Matrix devices made with cellulose or acrylic acid derivatives, which release the homogeneously dispersed drug based on the penetration of water through the matrix, have gained steady popularity because of their simplicity in design. The drawback of matrix-type delivery systems is their first-order drug delivery mechanism caused by changing surface area and drug diffusional path length with time. This drawback has been addressed by osmotic delivery systems, which maintain a zero-order drug release irrespective of the pH and hydrodynamics of the GI tract. Multiparticulate systems are gaining favor over single-unit dosage forms because of their desirable distribution characteristics, reproducible transit time, and reduced chance of gastric irritation owing to the localization of drug delivery.

Although several technologies for the production of microparticulate systems have been designed, thus far the mainstream technologies are still based on spray-drying, spheronization, and film-coating technology.

FDA regulation of oral Controlled-release drugs:

In the 1980s, FDA introduced rigorous regulations governing bioequivalence and in vitro–in vivo correlations for controlled-release products. Required pharmacokinetic evaluations involve

- relative bioavailability following single dose
- relative bioavailability following multiple doses
- effect of food
- dose proportionality
- unit dosage strength proportionality
- single-dose bioequivalence study

(Experimental versus marketed formulations at various strengths)

- In vivo–in vitro correlation
- Pharmacokinetic/pharmacodynamic (PK/PD) relationship.

In general, for drugs in which the exposure–response relationship has not been established or is unknown, applications for changing the formulation from immediate release to controlled release requires demonstration of the safety and efficacy of the product in the target patient population. When an NME is developed as a controlled-release dosage form, additional studies to characterize its absorption, distribution, metabolism, and excretion (ADME) characteristics are recommended.

The future of Drug Delivery System:

The future of controlled-release products is promising, especially in the following areas that present high promise and acceptability:

- Particulate systems: The micro particle and nanoparticle approach that involves biodegradable polymers and is aimed at the uptake of intact drug-loaded particles via the Peyer’s patches in the small intestine could be useful for delivery of peptide drugs that cannot, in general, be given orally.
- Chronopharmacokinetic systems: Oral controlled drug delivery with a pulsatile release regimen could effectively deliver drugs where need exists to counter naturally occurring processes such as bacterial/parasitical growth patterns (e.g., the once-daily oral Pulsys system introduced by Advancis Pharmaceutical Corp., which could potentially inhibit the emergence of resistant strains of microorganisms).
- Targeted drug delivery: Oral controlled drug delivery that targets regions in the GI tract and

releases drugs only upon reaching that site could offer effective treatment for certain disease states (e.g. colon-targeted delivery of antineoplastics in the treatment of colon cancer).

● Mucoadhesive delivery: This is a promising technique for buccal and sublingual drug delivery, which can offer rapid onset of action and superior bioavailability compared with simple oral delivery because it bypasses first—pass metabolism in the liver. (Das 2003)

Advantages of controlled drug delivery systems:

1. Improved patient convenience and compliance
2. Reduction in fluctuation in steady state levels.
3. Increased safety margin of high potency drugs.
4. Reduction in dose.
5. Reduction in health care cost.

Disadvantages of controlled drug delivery systems:

1. Decreased systemic availability
2. Poor invitro-invivo correlations
3. Chances of dose dumping
4. Dose withdrawal is not possible.
5. Higher cost of formulation

Applications of controlled drug delivery system:

1) Mucoadhesive drug delivery system
2) Colon drug delivery system
3) Pulmonary drug delivery system
4) Ocular drug delivery system
5) Oral thin films
6) Nasal drug delivery system
7) Gastro retentive drug delivery system
8) Vaginal drug delivery system
9) Resealed erythrocytes

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ORAL CONTROLLED DRUG DELIVERY SYSTEM

Bajaj Amruta, Katedeshmukh Ramesh

Introduction:

Oral drug delivery is the most widely utilized route of administration among all the routes [nasal, ophthalmic, rectal, transdermal and Parenteral routes] that have been explored for systemic delivery of drugs via pharmaceutical products of different dosage form. Oral route is considered most natural, uncomplicated, convenient and safe [in respect to Parenteral route] due to its ease of administration, patient acceptance, and cost-effective manufacturing process. Pharmaceutical products designed for oral delivery are mainly immediate release type or conventional drug delivery systems, which are designed for immediate release of drug for rapid absorption. These immediate release dosage forms have some limitations such as:

1) Drugs with short half-life requires frequent administration, which increases chances of missing dose of drug leading to poor patient compliance.

2) A typical peak-valley plasma concentration-time profile is obtained which makes attainment of steady state condition difficult.

3) The unavoidable fluctuations in the drug concentration may lead to under medication or overmedication as the CSS values fall or rise beyond the therapeutic range.

4) The fluctuating drug levels may lead to precipitation of adverse effects especially of a drug with small therapeutic index, whenever overmedication occurs.

In order to overcome the drawbacks of conventional drug delivery systems, several technical advancements have led to the development of controlled drug delivery system that could revolutionize method of medication and provide a number of therapeutic benefits. (Hemnani M. 2011)

TABLE 1:-Benefit Characteristics Of Oral Controlled-Release Drug Delivery System. (Das N. 2003)

Benefit	**Reason**
Therapeutic advantage	Reduction in drug plasma level fluctuations; maintenance of a steady plasma level of the drug over a prolonged time period, ideally simulating an intravenous infusion of a drug.
Reduction in adverse side effects and improvement in tolerability	Drug plasma levels are maintained within a narrow window with no sharp peaks and with AUC of plasma concentration versus time curve comparable with total AUC from multiple dosing with immediate release dosage forms. This greatly reduces the possibility of side effects, as the scale of side effects increase as we approach the MSC.
Patient comfort and compliance	Oral drug delivery is the most common and convenient for patients, and a reduction in dosing frequency enhances compliance.
Reduction in healthcare cost	The total cost of therapy of the controlled release product could be comparable or lower than the immediate-release product. With reduction in side effects, the overall expense in disease management also would be reduced.

Controlled Drug Delivery Systems: (Hemnani M. 2011)

Controlled drug delivery systems have been developed which are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and/or targeting the delivery of drug to a tissue. Controlled drug delivery or modified drug delivery systems are conveniently divided into four categories.

1) Delayed release
2) Sustained release

3) Site-specific targeting
4) Receptor targeting

More precisely, controlled delivery can be defined as:

1) Sustained drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects.

2) Localized drug action by spatial placement of a controlled release system adjacent to or in the diseased tissue.

3) Targeted drug action by using carriers or chemical derivatives to deliver drug to a particular target cell type.

4) Provide a physiologically/therapeutically based drug release system. In other words, the amount and the rate of drug release are determined by the physiological/ therapeutic needs of the body.

A controlled drug delivery system is usually designed to deliver the drug at particular rate. Safe and effective blood levels are maintained for a period as long as the system continues to deliver the drug. Controlled drug delivery usually results in substantially constant blood levels of the active ingredient as compared to the uncontrolled fluctuations observed when multiple doses of quick releasing conventional dosage forms are administered to a patient.

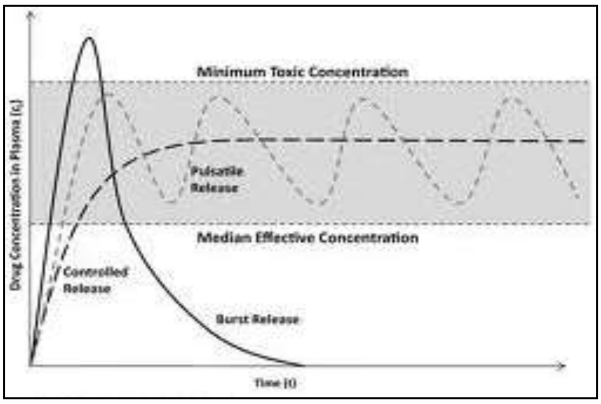

Figure 1: A hypothetical plasma concentration-time profile from conventional multiple dosing and single doses of sustained and controlled delivery formulations. Rationale of Controlled Drug Delivery

The basic rationale for controlled drug delivery is to alter the pharmacokinetics and pharmacodynamics of pharmacologically active moieties by using novel drug delivery system or by modifying the molecular structure and/or physiological parameters inherent in a selected route of administration. It is desirable that the duration of drug action become more a design property of a rate controlled dosage form, and less, or not at all, a property of the molecule's inherent kinetic properties. Thus optimal design of controlled release systems necessitates a thorough understanding of the pharmacokinetics and pharmacodynamics of the drug. The primary objectives of controlled drug delivery are to ensure safety and to improve efficacy of drugs as well as patient compliance. This is achieved by better control of plasma drug levels and less frequent dosing. The dose and dosing interval can be modified in case of conventional dosage forms. However, therapeutic window of plasma concentration below which no therapeutic effect is exhibited and above which undesirable effects are manifested. Therapeutic index is the prime parameter for development of a controlled delivery system of a particular drug candidate.

Factors Affecting the Design and Performance of Controlled Drug Delivery: (Hemnani M. 2011)

1. Drug Properties:
Partition coefficient
Drug stability
Protein binding
Molecular size and diffusivity

2. Biological Properties:
Absorption
Metabolism
Elimination and biological half life
Dose size
Route of administration
Target sites
Acute or chronic therapy
Disease condition

Advantages of Controlled Drug Delivery System: (Patel H. Nov Dec 2011):

1. Avoid patient compliance problems.
2. Employ less total drug

3. Minimize or eliminate local side effects
4. Minimize or eliminate systemic side effects
5. Obtain less potentiating or reduction in drug activity with chronic use.
6. Minimize drug accumulation with chronic dosing.

7. Improve efficiency in treatment

8. Cures or controls condition more promptly.
9. Improves control of condition i.e., reduced fluctuation in drug level.
10. Improves bioavailability of some drugs.
11. Make use of special effects, E.g. Sustained-release aspirin for morning relief of arthritis by dosing before bed time.

12. Economy i.e. reduction in health care costs. The average cost of treatment over an extended time period may be less, with less frequency of dosing, enhanced therapeutic benefits and reduced side effects.

13. The time required for health care personnel to dispense and administer the drug and monitor patient is also reduced.

Disadvantages: (Kamboj S 2009)

1) Decreased systemic availability in comparison to immediate release conventional dosage forms, which may be due to incomplete release, increased first-pass metabolism, increased instability, insufficient residence time for complete release, site specific absorption, pH dependent stability etc.
2) Poor *in vitro* – *in vivo* correlation.
3) Possibility of dose dumping due to food, physiologic or formulation variables or chewing or grinding of oral formulations by the patient and thus, increased risk of toxicity.
4) Retrieval of drug is difficult in case of toxicity, poisoning or hypersensitivity reactions.
5) Reduced potential for dosage adjustment of drugs normally administered in varying strengths.
6) Stability problems.
7) Increased cost.
8) More rapid development of tolerance and counseling.
9) Need for additional patient education and counseling

Oral Controlled Drug Delivery Systems: (Hemnani M. 2011)

Oral controlled release drug delivery is a drug delivery system that provides the continuous oral delivery of drugs at predictable and reproducible kinetics for a predetermined period throughout the course of GI transit and also the system that target the delivery of a drug to a specific region within the GI tract for either a local or systemic action.

All the pharmaceutical products formulated for systemic delivery via the oral route of administration, irrespective of the mode of delivery (immediate, sustained or controlled release) and the design of dosage form (either solid dispersion or liquid), must be developed within the intrinsic characteristics of GI physiology. Therefore the scientific framework required for the successful development of oral drug delivery systems consists of basic understanding of

- (i) physicochemical, pharmacokinetic and pharmacodynamic characteristics of the drug
- (ii) the anatomic and physiologic characteristics of the gastrointestinal tract
- (iii) physicochemical characteristics and the drug delivery mode of the dosage form to be designed.

The main areas of potential challenge in the development of oral controlled drug delivery systems are:-

1) Development of a drug delivery system: To develop a viable oral controlled release drug delivery system capable of delivering a drug at a therapeutically effective rate to a desirable site for duration required for optimal treatment.

2) Modulation of gastrointestinal transit time: To modulate the GI transit time so that the drug delivery system developed can be transported to a target site or to the vicinity of an absorption site and reside there for a prolonged period of time to maximize the delivery of a drug dose.

3) Minimization of hepatic first pass elimination: If the drug to be delivered is subjected to extensive hepatic first-pass elimination, preventive measures should be devised to either bypass or minimize the extent of hepatic metabolic effect.

Methods Used To Achieve Controlled Release Of Orally Administered Drugs:

A. Diffusion Controlled System:

Basically diffusion process shows the movement of drug molecules from a region of a higher concentration to one of lower concentration.
This system is of two types:

a) Reservoir type: A core of drug surrounded by polymer membrane, which controls the release rate, characterizes reservoir devices.

b) Matrix type: Matrix system is characterized by a homogenous dispersion of solid drug in a polymer mixture.

B. Dissolution Controlled Systems:

a) **Reservoir type:** Drug is coated with a given thickness coating, which is slowly dissolved in the contents of gastrointestinal tract. By alternating layers of drug with the rate controlling coats as shown in figure no.2, a pulsed delivery can be achieved. If the outer layer is quickly releasing bolus dose of the drug, initial levels of the drug in the body can be quickly established with pulsed intervals

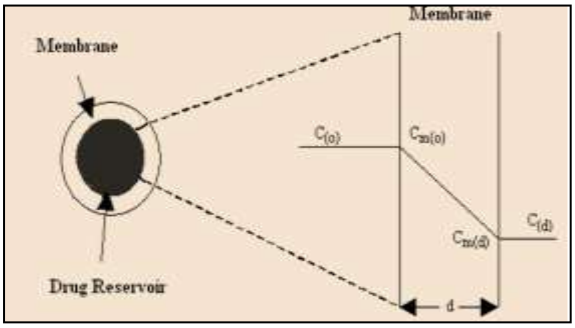

Figure 2: Schematic representation of diffusion controlled drug release reservoir system.

b) **Matrix type:** The more common type of dissolution controlled dosage form as shown in figure .3. It can be either a drug impregnated sphere or a drug impregnated tablet, which will be subjected to slow erosion.

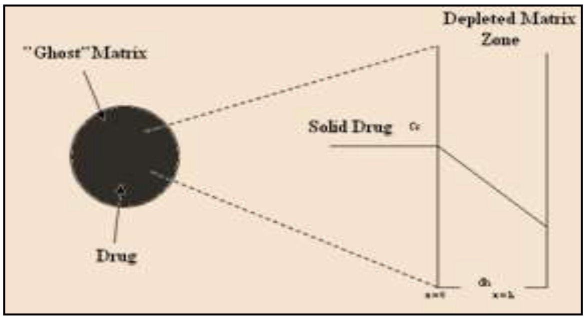

Figure 3: Schematic representation of diffusion controlled drug release matrix system.

C. Bioerodable and Combination of Diffusion and Dissolution Systems:

It is characterized by a homogeneous dispersion of drug in an erodible matrix. (Shown in figure.4)

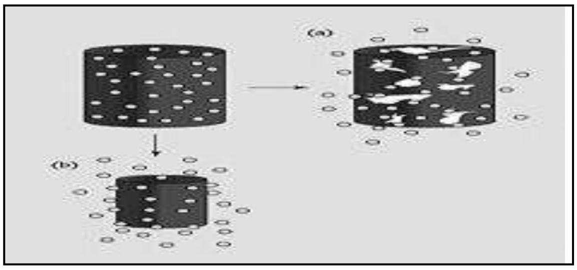

Figure 4: Drug delivery from (a) bulk-eroding and (b) surface-eroding Bio erodible systems.

D. **Methods using Ion Exchange:** It is based on the drug resin complex formation when an ionic solution is kept in contact with ionic resins. The drug from these complexes gets exchanged in gastrointestinal tract and released with excess of Na+ and Cl- present in gastrointestinal tract.

E. **Methods using osmotic pressure:** It is characterized by drug surrounded by semi permeable membrane and release governed by osmotic pressure.

F. **pH– Independent formulations:** A buffered controlled release formulation as shown in figure 5, is prepared by mixing a basic or acidic drug with one or more buffering agents, granulating with appropriate pharmaceutical excipients and coating with GI fluid permeable film forming polymer. When GI fluid permeates through the membrane the buffering agent adjusts the fluid inside to suitable constant pH thereby rendering a constant rate of drug release.

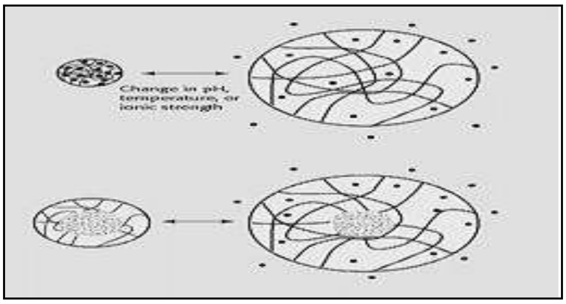

Figure 5: Drug delivery from environmentally pH sensitive release systems.

G. **Altered density formulations:** Several approaches have been developed to prolong the residence time of drug delivery system in the gastrointestinal tract.
High-density approach
Low-density approach

Matrix Tablet: (Patel H. Nov Dec 2011)

Advantages of matrix tablet:

- Easy to manufacture
- Versatile, effective and low cost
- Can be made to release high molecular weight compounds
- The sustained release formulations may maintain therapeutic concentrations over prolonged periods.
- The use of sustain release formulations avoids the high blood concentration.
- Sustain release formulations have the potential to improve the patient compliance.
- Reduce the toxicity by slowing drug absorption.
- Increase the stability by protecting the drug from hydrolysis or other derivative changes in gastrointestinal tract.
- Minimize the local and systemic side effects.
- Improvement in treatment efficacy.
- Minimize drug accumulation with chronic dosing.
- Usage of less total drug.
- Improvement the bioavailability of some drugs.
- Improvement of the ability to provide special effects. Ex: Morning relief of arthritis through bed time dosing.

Disadvantages of matrix tablet: (Patel H. Nov Dec 2011)
The remaining matrix must be removed after the drug has been released.
High cost of preparation.
The release rates are affected by various factors such as, food and the rate transit through the gut.
The drug release rates vary with the square root of time. Release rate continuously diminishes due to an increase in diffusional resistance and/or a decrease in effective area at the diffusion front. However, a substantial sustained effect can be produced through the use of very slow release rates, which in many applications are indistinguishable from zero-order.

Classification Of Matrix Tablets:
On the Basis of Retardant Material Used: Matrix tablets **can be divided in to 5 types.**

1. Hydrophobic Matrices (Plastic matrices):

The concept of using hydrophobic or inert materials as matrix materials was first introduced in 1959. In this method of obtaining sustained release from an oral dosage form, drug is mixed with an inert or hydrophobic polymer and then compressed in to a tablet. Sustained release is produced due to the fact that the dissolving drug has diffused through a network of channels that exist between compacted polymer particles. Examples of materials that have been used as inert or hydrophobic matrices include polyethylene, polyvinyl chloride, ethyl cellulose and acrylate polymers and their copolymers. The rate-controlling step in these formulations is liquid penetration into the matrix. The possible mechanism of release of drug in such type of tablets is diffusion. Such types of matrix tablets become inert in the presence of water and gastrointestinal fluid.

2. Lipid Matrices:
These matrices prepared by the lipid waxes and related materials. Drug release from such matrices occurs through both pore diffusion and erosion. Release characteristics are therefore more sensitive to digestive fluid composition than to totally insoluble polymer matrix. Carnauba wax in combination with stearyl alcohol or stearic acid has been utilized for retardant base for many sustained release formulation.

3. Hydrophilic Matrices:

Hydrophilic polymer matrix systems are widely used in oral controlled drug delivery because of their flexibility to obtain a desirable drug release profile, cost effectiveness, and broad regulatory acceptance. The formulation of the drugs in gelatinous capsules or more frequently, in tablets, using hydrophilic polymers with high gelling capacities as base excipients is of particular interest in the field of controlled release. Infect a matrix is defined as well mixed composite of one or more drugs with a gelling agent (hydrophilic polymer). These systems are called swellable controlled release systems. The polymers used in the preparation of hydrophilic matrices are divided in to three broad groups,

A. Cellulose derivatives: Methylcellulose 400 and 4000cPs, HEC; HPMC 25, 100, 4000 and 15000cPs; and Sodium carboxymethylcellulose.

B. Non cellulose natural or semi synthetic polymers: Agar-Agar; Carob gum; Alginates; Molasses; Polysaccharides of mannose and galactose, Chitosan and Modified starches.

4. Biodegradable Matrices: These consist of the polymers which comprised of monomers linked to one another through functional groups and have unstable linkage in the backbone. They are biologically degraded or eroded by enzymes generated by surrounding living cells or by nonenzymetic process in to oligomers and monomers that can be metabolized or excreted. Examples are natural polymers such as proteins and polysaccharides; modified natural polymers; synthetic polymers such as aliphatic poly (esters) and poly anhydrides.

5. Mineral Matrices: These consist of polymers which are obtained from various species of seaweeds. Example is Alginic acid which is a hydrophilic carbohydrate obtained from species of brown seaweeds (Phaephyceae) by the use of dilute alkali.

On the Basis of Porosity of Matrix: Matrix system can also be classified according to their porosity and consequently, Macro porous; Micro porous and Non-porous systems can be identified:

1. Macro porous Systems: In such systems the diffusion of drug occurs through pores of matrix, which are of size range 0.1 to 1 μm. This pore size is larger than diffusant molecule size.

2. Micro porous System: Diffusion in this type of system occurs essentially through pores. For micro porous systems, pore size ranges between 50 – 200 A°, which is slightly larger than diffusant molecules size.

3. Non-porous System: Non-porous systems have no pores and the molecules diffuse through the network meshes. In this case, only the polymeric phase exists and no pore phase is present.

Polymers used in matrix tablet:

Hydrogels: Polyhydroxyethylemethylacrylate (PHEMA), Cross-linked polyvinyl alcohol (PVA), Cross-linked polyvinyl pyrrolidone (PVP), Polyethylene oxide (PEO), Polyacrylamide (PA)

Soluble polymers: Polyethyleneglycol (PEG), polyvinyl alcohol (PVA), Polyvinylpyrrolidone (PVP), Hydroxypropyl methyl cellulose (HPMC)

Biodegradable polymers: Polylactic acid (PLA), Polyglycolic acid (PGA), Polycaprolactone (PCL), Polyanhydrides, Polyorthoesters

Non-biodegradable polymers: Polyethylene vinyl acetate (PVA), Polydimethylsiloxane (PDS), Polyether urethane (PEU), Polyvinyl chloride (PVC), Cellulose acetate (CA), Ethyl cellulose (EC)

Mucoadhesive polymers: Polycarbophil, Sodium carboxymethyl cellulose, Polyacrylic acid, Tragacanth, Methyl cellulose, Pectin

Natural gums: Xanthan gum, Guar gum, Karaya gum, Locust bean gum

Components of matrix tablets: (ME. 2005)
These include:

- Active drug
- Release controlling agent(s): matrix formers
- Matrix Modifiers, such as channelling agents and wicking agents
- Solubilizers and pH modifiers
- Lubricants and flow aid
- Supplementary coatings to extend lag time further reduce drug release etc.
- Density modifiers (if required)

Mechanism Of Drug Release From Matrix Tablet:

Drug in the outside layer exposed to the bathing solution is dissolved first and then diffuses out of the matrix. This process continues with the interface between the bathing solution and the solid drug moving toward the interior. It follows that for this system to be diffusion controlled, the rate of dissolution of drug particles within the matrix must be much faster than the diffusion rate of dissolved drug leaving the matrix. Derivation of the mathematical model to describe this system involves the following assumptions:

a) A pseudo-steady state is maintained during drug release,

b) The diameter of the drug particles is less than the average distance of drug diffusion through the matrix,

c) The bathing solution provides sink conditions at all times.

The release behaviour for the system can be mathematically described by the following equation:

$dM/dh = Co. dh - Cs/2$ ……………… (1)

Where, dM = Change in the amount of drug released per unit area

dh = Change in the thickness of the zone of matrix that has been depleted of drug

Co = Total amount of drug in a unit volume of matrix

Cs = Saturated concentration of the drug within the matrix.

Additionally, according to diffusion theory:

$dM = (Dm. Cs / h)\, dt$.......................... (2)

Where, Dm = Diffusion coefficient in the matrix.

h = Thickness of the drug-depleted matrix

dt = Change in time

By combining equation 1 and equation 2 and integrating:

$M = [Cs. Dm (2Co - Cs)\, t]^{1/2}$ ……………… (3)

When the amount of drug is in excess of the saturation concentration then:

$M = [2Cs.Dm.Co.t]^{1/2}$ ……………………… (4)

Equation 3 and equation 4 relate the amount of drug release to the square-root of time. Therefore, if a system is predominantly diffusion controlled, then it is expected that a plot of the drug release vs. square root of time will result in a straight line. Drug release from a porous monolithic matrix involves the simultaneous penetration of surrounding liquid, dissolution of drug and leaching out of the drug through tortuous interstitial channels and pores. The volume and length of the openings must be accounted for in the drug release from a porous or granular matrix:

$M = [Ds.\ Ca.\ p/T.\ (2Co - p.Ca)\, t]^{1/2}$ ……………. (5)

Where, p = Porosity of the matrix

t = Tortuosity Ca = solubility of the drug in the release medium

Ds = Diffusion coefficient in the release medium.

T = Diffusional path length For pseudo steady state,

the equation can be written as:

$M = [2D.Ca\ .Co\ (p/T)\ t]^{1/2}$ ……………………….. (6)

The total porosity of the matrix can be calculated with the following equation:

$p = pa + Ca/\rho + Cex / \rho ex$ ……………………… (7)

Where, p = Porosity

ρ = Drug density

pa = Porosity due to air pockets in the matrix

ρex = Density of the water soluble excipients

Cex = Concentration of water soluble excipients

For the purpose of data treatment, equation 7 can be reduced to: **$M = k.\ t^{1/2}$ ……………………….. (8)**

Where, k is a constant, so that the amount of drug released versus the square root of time will be linear, if the release of drug from matrix is diffusion-controlled. If this is the case, the release of drug from a homogeneous matrix system can be controlled by varying the following parameters:

- Initial concentration of drug in the matrix
- Porosity
- Tortuosity
- Polymer system forming the matrix
- Solubility of the drug.

Effect Of Release Limiting Factor On Drug Release:

The mechanistic analysis of controlled release of drug reveals that partition coefficient; diffusivity; diffusional path thickness and other system parameters play various rate determining roles in the controlled release of drugs from either capsules, matrix or sandwich type drug delivery systems.

A. **Polymer hydration:** It is important to study polymer hydration/swelling process for the maximum number of polymers and polymeric combinations. The more important step in polymer dissolution include absorption/adsorption of water in more accessible places, rupture of polymer-polymer linking with the simultaneous forming of water-polymer linking, separation of polymeric chains, swelling and finally dispersion of polymeric chain in dissolution medium

B. Drug solubility: Molecular size and water solubility of drug are important determinants in the release of drug from swelling and erosion controlled polymeric matrices. For drugs with reasonable aqueous solubility, release of drugs occurs by dissolution in infiltrating medium and for drugs with poor solubility release occurs by both dissolution of drug and dissolution of drug particles through erosion of the matrix tablet.

C. Solution solubility: In view of in vivo (biological) sink condition maintained actively by hem perfusion, it is logical that all the in vitro drug release studies should also be conducted under perfect sink condition. In this way a better simulation and correlation of in vitro drug release profile with in vivo drug administration can be achieved. It is necessary to maintain a sink condition so that the release of drug is controlled solely by the delivery system and is not affected or complicated by solubility factor.

D. Polymer diffusivity: The diffusion of small molecules in polymer structure is energy activated process in which the diffusant molecules moves to a successive series of equilibrium position when a sufficient amount of energy of activation for diffusion Ed has been acquired by the diffusant is dependent on length of polymer chain segment, cross linking and crystallinity of polymer. The release of drug may be attributed to the three factors viz,

i. Polymer particle size
ii. Polymer viscosity
iii. Polymer concentration.

i. Polymer particle size: Malamataris stated that when the content of hydroxyl propyl methylcellulose is higher, the effect of particle size is less important on the release rate of propranolol hydrochloride, the effect of this variable more important when the content of polymer is low. He also justified these results by considering that in certain areas of matrix containing low levels of hydroxyl propyl methylcellulose led to the burst release.

ii. Polymer viscosity: With cellulose ether polymers, viscosity is used as an indication of matrix weight. Increasing the molecular weight or viscosity of the polymer in the matrix formulation increases the gel layer viscosity and thus slows drug dissolution. Also, the greater viscosity of the gel, the more resistant the gel is to dilution and erosion, thus controlling the drug dissolution.

iii. Polymer concentration: An increase in polymer concentration causes an increase in the viscosity of gel as well as formulation of gel layer with a longer diffusional path. This could cause a decrease in the effective diffusion coefficient of the drug and therefore reduction in drug release. The mechanism of drug release from matrix also changes from erosion to diffusion as the polymer concentration increases.

E. Thickness of polymer diffusional path: The controlled release of a drug from both capsule and matrix type polymeric drug delivery system is essentially governed by Fick's law of diffusion:

JD = D dc/dx

Where, JD is flux of diffusion across a plane surface of unit area

D is diffusibility of drug molecule, dc/dx is concentration gradient of drug molecule across a diffusion path with thickness dx.

F. Thickness of hydrodynamic diffusion layer: It was observed that the drug release profile is a function of the variation in thickness of hydrodynamic diffusion layer on the surface of matrix type delivery devices. The magnitude of drug release value decreases on increasing the thickness of hydrodynamic diffusion layer δd.

G. Drug loading dose: The loading dose of drug has a significant effect on resulting release kinetics along with drug solubility. The effect of initial drug loading of the tablets on the resulting release kinetics is more complex in case of poorly water soluble drugs, with increasing initial drug loading the relative release rate first decreases and then increases, whereas, absolute release rate monotonically increases. In case of freely water soluble drugs, the porosity of matrix upon drug depletion increases with increasing initial drug loading. This effect leads to increased absolute drug transfer rate. But in case of poorly water soluble drugs another phenomenon also has to be taken in to account. When the amount of drug present at certain position within the matrix, exceeds the amount of drug soluble under given conditions, the excess of drug has to be considered as non-dissolved and thus not available for diffusion. The solid drug remains within tablet, on increasing the initial drug loading of poorly water soluble drugs, the excess of drug remaining with in matrix increases.

H. Surface area and volume: The dependence of the rate of drug release on the surface area of drug delivery device is well known theoretically and experimentally. Both the *in vitro* and *in vivo* rate of the drug release, are observed to be dependent upon surface area of dosage form. *Siepman et al.* found that release from small tablet is faster than large cylindrical tablets.

I. Diluent's effect: The effect of diluent or filler depends upon the nature of diluent. Water soluble diluents like lactose cause marked increase in drug release rate and release mechanism is also shifted towards Fickian diffusion; while insoluble diluents like dicalcium phosphate reduce the Fickian diffusion and increase the relaxation (erosion) rate of matrix. The reason behind this is that water soluble filler in matrices stimulate the water penetration in to inner part of matrix, due to increase in hydrophilicity of the system, causing rapid diffusion of drug, leads to increased drug release rate.

J. Additives: The effect of adding non-polymeric excipients to a polymeric matrix has been claimed to produce increase in release rate of hydro soluble active principles. These increases in release rate would be marked if the excipients are soluble like lactose and less important if the excipients are insoluble like tricalcium phosphate

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Biological Factors Influencing Release from Matrix Tablet:

Biological half-life.
Absorption.
Metabolism
Distribution
Protein binding
Margin of safety

Biological half-life: The usual goal of an oral SR product is to maintain therapeutic blood levels over an extended period of time. To achieve this, drug must enter the circulation at approximately the same rate at which it is eliminated. The elimination rate is quantitatively described by the half-life (t1/2). Each drug has its own characteristic elimination rate, which is the sum of all elimination processes, including metabolism, urinary excretion and all over processes that permanently remove drug from the blood stream. Therapeutic compounds with short half-life are generally are excellent candidate for SR formulation, as this can reduce dosing frequency. In general, drugs with half-life shorter than 2 hours such as furosemide or levodopa are poor candidates for SR preparation. Compounds with long half-lives, more than 8 hours are also generally not used in sustaining form, since their effect is already sustained. Digoxin and phenytoin are the examples.

Absorption: Since the purpose of forming a SR product is to place control on the delivery system, it is necessary that the rate of release is much slower than the rate of absorption. If we assume that the transit time of most drugs in the absorptive areas of the GI tract is about 8-12 hours, the maximum half-life for absorption should be approximately 3-4 hours; otherwise, the device will pass out of the potential absorptive regions before drug release is complete. Thus corresponds to a minimum apparent absorption rate constant of 0.17-0.23h-1 to give 80-95% over this time period. Hence, it assumes that the absorption of the drug should occur at a relatively uniform rate over the entire length of small intestine. For many compounds this is not true. If a drug is absorbed by active transport or transport is limited to a specific region of intestine, SR preparation may be disadvantageous to absorption. One method to provide sustaining mechanisms of delivery for compounds tries to maintain them within the stomach. This allows slow release of the drug, which then travels to the absorptive site. These methods have been developed as a consequence of the observation that co-administration results in sustaining effect. One such attempt is to formulate low density pellet or capsule. Another approach is that of bio adhesive materials.

Metabolism: Drugs those are significantly metabolized before absorption, either in the lumen or the tissue of the intestine, can show decreased bioavailability from slower-releasing dosage form.

Hence criteria for the drug to be used for formulating Sustained-Release dosage form is,

Drug should have law half-life (<5 hrs.)

Even a drug that is poorly water soluble can be formulated in SR dosage form. For the same, the solubility of the drug should be increased by the suitable system and later on that is formulated in the SR dosage form. But during this the crystallization of the drug, that is taking place as the drug is entering in the systemic circulation, should be prevented and one should be cautious for the prevention of the same.

Distribution: Drugs with high apparent volume of distribution, which influence the rate of elimination of the drug, are poor candidate for oral SR drug delivery system e.g. Chloroquine.

Protein Binding: The Pharmacological response of drug depends on unbound drug concentration drug rather than total concentration and all drug bound to some extent to plasma and or tissue proteins. Proteins binding of drug play a significant role in its therapeutic effect regardless the type of dosage form as extensive binding to plasma increase biological half-life and thus sometimes drug delivery system drug.

DRUGS USED	CATEGORY	METHOD USED	POLYMER USED
Zidovudine	Anti-viral	Direct Compression	HPMC-K4M, Carbopol-934, EC
Venlafexine	Anti-depressant	Wet Granulation	Beeswax, Carnauba wax
Domperidone	Anti-emetic	Wet Granulation	HPMC-K4M, Carbopol-934
Alfuzosin	Alfa-adrenergic Agonist	Direct Compression	HPMC-K15M, Eudragit-RSPO
Minocycline	Antibiotic	Wet Granulation	HPMC-K4M, HPMC-K15M, EC
Ibuprofen	Anti-inflammatory	Wet Granulation	EC, CAP
Metformin HCL	Anti-diabetic	Direct Compression	HPMC-K100M, EC
Propranolol HCL	Beta-adrenergic blocker	Wet Granulation	Locust bean gum, HPMC
Furosemide	Anti-diuretic	Direct Compression	Guar gum, Pectin, Xanthan gum
Acarbose	Anti-diabetic	Direct Compression	HPMC, Eudragit
Aceclofenac	Anti-inflammatory	Wet Granulation	HPMC-K4M,K15M, K100M,E15,EC, Guar gum
Ambroxol HCL	Expectorent, Mucolytic	Direct Compression	HPMC-K100M,
Aspirin	Anti-inflammatory	Direct Compression	EC, Eudragit-RS100, S100
Diclofenac Na	Anti-inflammatory	Wet Granulation	Chitoson, EC, HPMCP, HPMC
Diethylcarbamazepine citrate	Anti-filarial	Wet Granulation	Guar gum, HPMC-E15LV
Diltiazem	Ca+2 channel blocker	Direct Compression	HPMC-K100M, HPMC-K4M, Karaya gum, Locust bean gum, Sod.CMC
Enalpril meleate	ACE inhibitor	Direct Compression	HPMC-K100M,HPMC K4M,
Flutamide	Anti-androgen	Direct Compression	HPMC-K4M, Sod.CMC, Guar gum, Xanthan gum
Indomethacin	Anti-inflammatory	Direct Compression	EC, HPMC
Chlorphenarimine meleate	H1 antagonist	Melt-extrusion	Xanthan gum,Chitoson
Itopride HCL	Prokinetic agent	Direct Compression	HPMC-K100M, HPMC-K4M, EC
Losartan potassium	Anti-hypertensive	Direct Compression	HPMC-K100M, HPMC-K4M, Eudragit-RSPO
Metoclopromide	Anti-emetic	Direct Compression / Wet Granulation	HPMC, CMC, EC, SSG
Miconazole	Anti-fungal	Direct Compression / Wet Granulation	Pectin, HPMC
Naproxen	Morphine antagonist	Direct Compression	HPMC-K100M, HPMC-K15M, PVP
Nicorandil	Ca+2 channel blocker	Wet Granulation	HPMC, CMC, EC
Ondansertan	Anti-hypertensive	Wet Granulation	HPMC-K100M, HPMC-K4M, HPMC-K15M
Phenytoin Na	Anti-epileptic	Wet Granulation	Tragacanth, Acacia, Guar gum, Xanthan gum
Ranitidine HCL	H2 antagonist	Direct Compression	Chitoson, Carbopol-940
Theophylline	Respiratory depressant	Direct Compression	Carbopol-934P, HPMC-K100M, HPMC-K4M, HPMC-K15M, EC

Osmotic Drug Delivery:

Introduction (M. 2009)

Controlled release dosage form are designed to release drug in-vivo according to predictable rate that can be verified by in-vitro measurement Potential development and new approaches to oral controlled release dosage form includes,

1. Hydrodynamic pressure controlled system
2. Intragastric floating tablet
3. Transmucosal tablet
4. Microporous membrane coated tablet.

Advantages: (Ghosh T. 2011)

- Decrease frequency of dosing.
- Reduce the rate of rise of drug concentration in the body.
- Delivery may be pulsed or desired if required.
- Delivery ratio is independent of pH of the environment.
- Delivery is independent of hydrodynamic condition, this suggest that drug delivery is independent of G.I. motility.
- Sustained and consistence blood level of drug within the therapeutic window.
- Improve patient compliance.
- High degree of in vitro- in vivo correlation is obtained in osmotic system.
- Reduce side effect.
- Delivery rate is also independent of delivery orifice size within the limit.

Disadvantage & limitation (Ghosh T. 2011)

OCODDS have produced significant clinical benefit invarious therapeutic areas .Some system have enhanced patient compliance, while other has minimized the side effect of their active compounds. However some limitations of OCODDS have been reported.

- Slightly higher cost of good than matrix tablet or multiparticulates ion capsule dosage form.
- Gastro intestinal obstruction cases have been observed with the patient receiving Nifedipine GITS tablet.
- Another case was reported for osmosin (Indomethacin OROS) which was first introduced in the United Kingdom in 1983 .A few month later after its introduction frequent incidences of serious gastrointestinal reaction was observed leading to osmosin withdrawal. Various explanations were given based on the toxic effect of KCl used in osmosin.
- Magnetic resonance imaging (MRI) of tablet elucidate that nonuniform coating leads to different pattern of drug release among the batches.

What Is Osmotic Pressure: (M. 2009)

Osmosis

Osmosis can be defined as the net movement of water across a selectively permeable membrane driven by a difference in osmotic pressure across the membrane. It is driven by a difference in solute concentrations across the membrane that allows passage of water, but rejects most solute molecules or ions. **Osmotic pressure** is the pressure which, if applied to the more concentrated solution, would prevent transport of water across the semipermeable membrane.

The first osmotic effect was reported by Abbe Nollet in 1748. Later in 1877, Pfeffer performed an experiment using semi-permeable membrane to separate sugar solution from pure water. He showed that the osmotic pressure of the sugar solution is directly proportional to the solution concentration and the absolute temperature. In 1886, Vant Hoff identified an underlying proportionality between osmotic pressure, concentration and temperature. He revealed that osmotic pressure is proportional to concentration and temperature and the relationship can be described by following equation.

$\Pi = Ø c RT$

Where, p = Osmotic pressure

Π = osmotic coefficient

c = molar concentration

R = gas constant

T = Absolute temperature

Osmotic pressure is a colligative property, which depends on concentration of solute that contributes to osmotic pressure. Solutions of different concentrations having the same solute and solvent system exhibit an osmotic pressure proportional to their concentrations. Thus a constant osmotic pressure, and thereby a constant influx of water can be achieved by an **osmotic** delivery system that results in a constant zero order release rate of drug.

Osmotically controlled drug delivery systems

Osmotic pressure is used as driving force for these systems to release the drug in controlled manner. **Osmotic** drug delivery technique is the most interesting and widely acceptable among all other technologies used for the same. Intensive research has been carried out on osmotic systems and several patents are also published. Development of osmotic drug delivery systems was pioneered by **Alza** and it holds major number of the patents analyzed and also markets several products based on osmotic principle. These systems can be used for both route of administration i.e. oral and parenterals. Oral **osmotic** systems are known as gastro-intestinal therapeutic systems (GITS). Parenteral **osmotic** drug delivery includes implantable pumps.

Historical aspects of osmotic pumps

About 75 years after discovery of the osmosis principle, it was first used in the design of drug delivery systems.[5] Rose and Nelson, the Australian scientists, were initiators of osmotic drug delivery. In 1955, they developed an implantable pump, which consisted of three chambers: a drug chamber, a salt chamber contains excess solid salt, and a water chamber. The drug and water chambers are separated by rigid semipermeable membrane. The difference in osmotic pressure across the membrane moves water from the water chamber into the salt chamber. The volume of the salt chamber increases because of this water flow, which distends the latex diaphragm separating the salt and drug chambers, thereby pumping drug out of the device. The design and mechanism of this pump is comparable to modern push-pull osmotic pump. The major disadvantage of this pump was the water chamber, which must be charged before use of the pump. The pumping rate of this push-pull pump is given by the equation.

$dM/dt = dV/dt \times c$

In general, this equation, with or without some modifications, applies to all other type of osmotic systems.

Several simplifications in Rose-Nelson pump were made by Alza Corporation in early 1970s. The Higuchi-Leeper pump is modified version of Rose-Nelson pump. It has no water chamber, and the device is activated by water imbibed from the surrounding environment. The pump is activated when it is swallowed or implanted in the body. This pump consists of a rigid housing, and the semipermeable membrane is supported on a perforated frame. It has a salt chamber containing a fluid solution with excess solid salt. Recent modification in Higuchi-Leeper pump accommodated pulsatile drug delivery. The pulsatile release was achieved by the production of a critical pressure at which the delivery orifice opens and releases the drug.

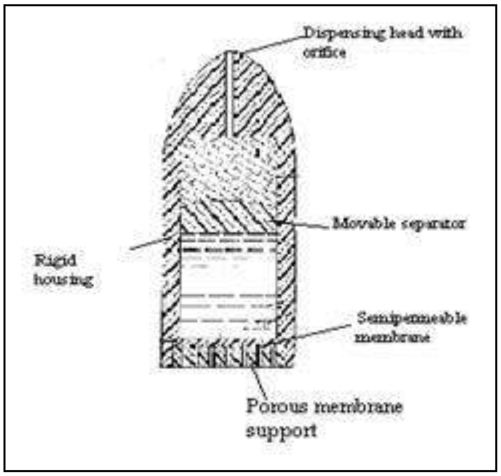


Fig. 6: Higuchi-Leeper pump

Further simplified variant of Rose-Nelson pump was developed by Higuchi and Theeuwes. This pump comprises a rigid, rate controlling outer semipermeable membrane surrounding a solid layer of salt coated on the inside by an elastic diaphragm and on the outside by the membrane. In use, water is osmotically drawn by the salt chamber, forcing drug from the drug chamber.

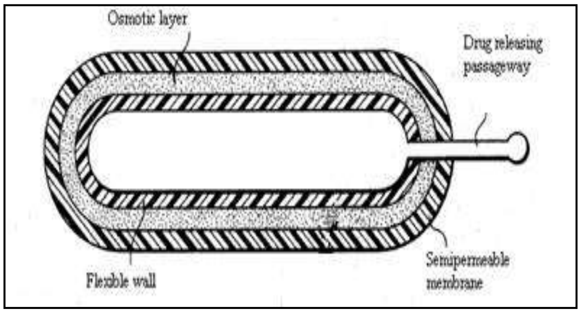


Fig. 7: Higuchi-Theeuwes pump

In 1975, the major leap in **osmotic** delivery occurred as the elementary **osmotic** pump for oral delivery of drugs was introduced. The pump consists of an **osmotic** core containing the drug, surrounded by a semipermeable membrane with a delivery orifice. When this pump is exposed to water, the core imbibes water osmotically at a controlled rate, determined by the membrane permeability to water and by the osmotic pressure of the core formulation. As the membrane is non-expandable, the increase in volume caused by the imbibition of water leads to the development of hydrostatic pressure inside the tablet. This pressure is relieved by the flow of saturated solution out of the device through the delivery orifice. This process continues at a constant rate until the entire solid agent inside the tablet has been dissolved and only a solution filled coating membrane is left. This residual dissolved agent continues to be delivered at a declining rate until the osmotic pressure inside and outside the tablet is equal. Normally, the EOP delivers 60-80% of its contents at a constant rate, and there is a short lag time of 30-60 min as the system hydrates before zero order delivery from the EOP is obtained.[8]

Apart from oral **osmotic** pumps, the development of miniature **implantable osmotic pumps** in the mid-1970s was a major breakthrough to deliver wide range of drugs and hormones, including peptides at constant and programmed rate in mice, rat and larger animals. These implants provide a convective stream of drug solution that can be directed through suitable catheter connections to sites in the animal remote from itself. Most recently the implantable pumps for human use are developed to deliver the drug for targeting or systemic application.

Classification of Osmotic drug delivery systems:

Many forms of **osmotic** pumps are reported in the literature but, in general they can be divided in oral and implantable systems. The detailed information on various **osmotic** systems is classified in **Table 2**.

Oral osmotic drug delivery systems (Ghosh T. 2011)

As oral route is the most popular route of administration, most of the **osmotic** systems are developed as oral drug delivery. It is possible to deliver APIs at zero-order release rate, independent of gastric pH and hydrodynamic conditions with these osmotically controlled drug delivery systems.

The OCODDS can be conveniently classified in to following types:

- **Single chamber osmotic pump**

1. Elementary osmotic pump

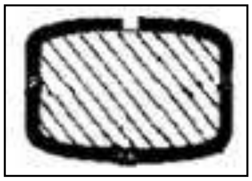
- **Multi chamber osmotic pump**

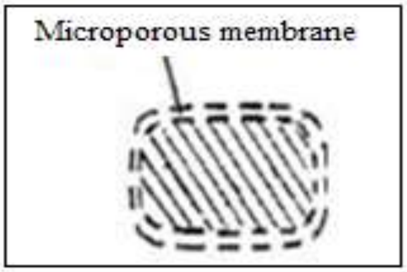
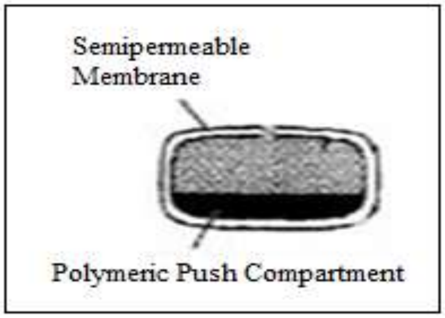
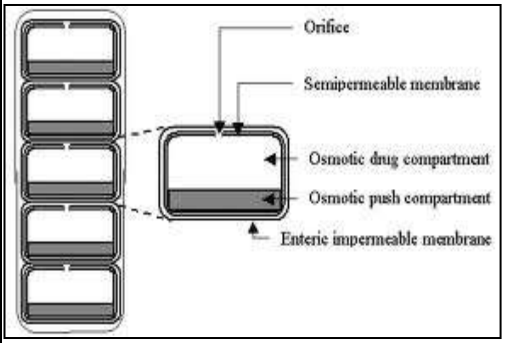
1. Push pull osmotic pump.
2. Osmotic pump with non expanding second chamber.

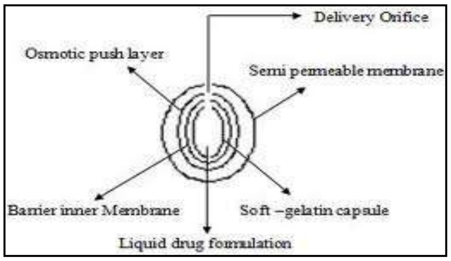
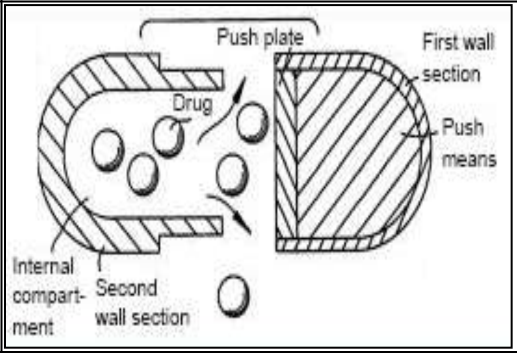
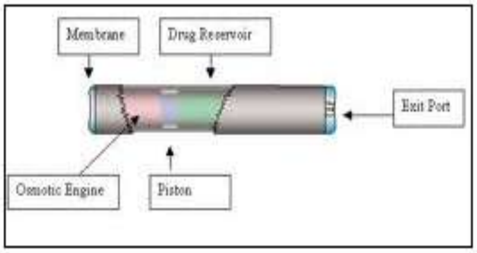
- **Specific types**

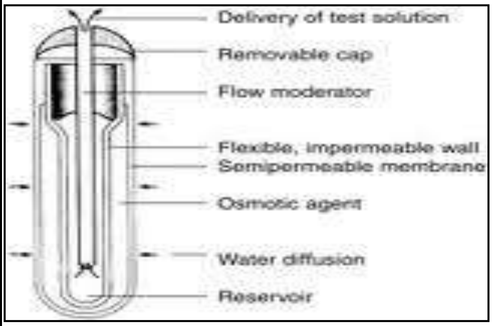
1. Controlled porosity osmotic pump.
2. Monolithic osmotic systems.
3. Osmotic bursting osmotic pump.
4. OROS – CT
5. Multi particulate delayed release systems (MPDRS)
6. Liquid Oral Osmotic System.(L-OROS)

Table 2: Different types of osmotic systems-Design, mechanism and uses (M. 2009)

Osmotic System	**Design of Dosage Form**	**Mechanism**	**Applications & Diagram**
Oral osmotic tablets			
Single chamber osmotic pumps			
Elementary Osmotic Pump (EOP)	**Core:** API osmogents **Coat:** Semi permeable membrane with delivery orifice.	The water penetrates inside the dosage form at the rate determined by the fluid permeability of the membrane and osmotic pressure of core formulation.	**Moderately soluble** API - 60-80% constant release 

Controlled-porosity osmotic pump (CPOP)	**Core:** API ± osmogents **Coat:** Semi permeable membrane with water soluble additives	Water-soluble additives dissolve after coming in contact with water, resulting in an in situ formation of a microporous membrane. The resulting membrane is substantially permeable to both water and dissolved solutes and the mechanism of drug release was found to be osmotic.	
Osmotic bursting osmotic pump	**Core:** API ± osmogents **Coat:** Semi permeable membrane without delivery orifice	When placed in aqueous environment, water is imbibed and hydraulic pressure is built up inside the system, then wall ruptures and the contents are released.	For pulsated release
Multi-chamber osmotic pumps			
Push-pull osmotic pump (PPOP)	**Core Tablet:** Layer 1: API ± osmogents Layer 2: Polymeric osmotic agents **Coat:** Semi permeable membrane with delivery orifice	After coming in contact with the aqueous environment, polymeric osmotic layer swells and pushes the drug layer, and thus releasing drug in the form of fine dispersion via the orifice.	· For delivery of APIs having **extremes of water solubility** · Modifications can be done: delayed push-pull , multi-layer push-pull push-stick system 
Oral osmotic capsules			
OROS-CT	Single osmotic unit or a unit containing as many as five to six PPOP filled in hard gelatin capsule. The osmotic system is enteric coated.	Gelatin capsule shell dissolves after coming in contact with GI fluids. Enteric coating on the system prevents entry of fluid from stomach to the system and it dissolves after entering into intestine. The water imbibes into the core and push compartment will swell. At the same time, the flowable gel is formed which is pushed out via delivery port	· For colon-targeting and can be used as local or systemic therapy.  Orifice Semipermeable membrane Osmotic drug compartment Osmotic push compartment Enteric impermeable membrane

L-OROS (Soft-Cap & Hard-Cap)	Liquid API formulation is present in a soft gelatin capsule, which is surrounded with the barrier layer, the osmotic layer, and the release rate-controlling membrane. A delivery orifice is formed through these three layers.	When the system comes in contact with aqueous environment, water permeates across the rate controlling membrane and activates the osmotic layer. The expansion of the osmotic layer results in the development of hydrostatic pressure inside the system, thereby forcing the liquid formulation to break through the hydrated gelatin capsule shell at the delivery orifice.	· To deliver APIs as liquid formulations and combine the benefits of extended release with high bioavailability. · Suitable for controlled delivery of **lipophilic APIs**  Delivery Orifice Osmotic push layer Semi permeable membrane Barrier inner Membrane Soft –gelatin capsule Liquid drug formulation
Pelleted delayed release	Multi-particulate delayed release systems consist of pellets of API (with or without osmogents) coated with SPM.	Rapid expansion of membrane after coming in contact with aqueous environment resulting in pore-formation and API release	· High flux rates and thus having higher release rates for **poorly water-soluble** APIs
Asymmetric membrane capsule	Capsule wall made up of water insoluble semipermeable polymer	Imbibition of water through the capsule wall and dissolving soluble components within it and releasing from same wall	· High water permeability and controlled porosity
Telescopic capsule for delayed release	This device consists of two chambers, the first contains the drug and an exit port, and the second contains osmotic engine. Layer of wax-like material separates the two sections.	As fluid is imbibed the housing of the dispensing device, the osmotic engine expand and exerts pressure on the slidable connected first and second wall sections.	 Push plate First wall section Drug Push means Internal compart-ment Second wall section
Implantable osmotic systems			
Duros osmotic pump	Implantable drug-dispensing osmotic pump, shaped as a small rod with titanium housing.	Through osmosis, water from the body is slowly drawn through the semi-permeable membrane into the pump by osmotic agent residing in the engine compartment, which expands the osmotic agent and displaces a piston to dispense small amounts of drug formulation from the drug reservoir through the orifice.	·Systemic or site-specific administration of a drug  Membrane Drug Reservoir Exit Port Osmotic Engine Piston

Alzet osmotic pump	Empty reservoir within the core of the pump is filled with the drug or hormone solution to be delivered and is surrounded by salt chamber with impermeable layer between them.	Water enters into the salt chamber through semipermeable membrane and causes compression of flexible reservoir and delivery of drug solution.	· To deliver drugs, hormones, and other test agents continuously at controlled rates from one day to six weeks  Delivery of test solution Removable cap Flow moderator Flexible, impermeable wall Semipermeable membrane Osmotic agent Water diffusion Reservoir

General mechanism for drug release from osmotic pumps (M. 2009)

As described earlier, the basic equation which applies to osmotic systems is

$dM / dt = dV / dt \times c$

Where,

dM / dt= mass release

dV / dt= volumetric pumping rate

c = concentration of drug

But,

$dV / dt = (A/ h)L_p (\sigma \Delta\Pi - \Delta p)$

Where,

A= membrane area

h= thickness of membrane

L_p= mechanical permeability

σ =reflection coefficient

$\Delta\Pi$=osmotic pressure difference

Δp = hydrostatic pressure difference

As the size of orifice delivery increases, Δp decrease, so $\Delta\Pi >> \Delta p$ and equation becomes

$dV / dt = A/ h \, L_p (\sigma \Delta\Pi)$

When the **osmotic** pressure of the formulation is large compared to the **osmotic** pressure of the environment, p can be substituted for Dp.

$dV / dt = A / h \, L_p \, \sigma\Pi = A / hk \, \Pi$ ($k = L_p\sigma$ = membrane permeability)

Now, equation (1) can be given as

$dM / dt = (A / h) \, k \, \Pi \, c = (A / h) \, k \, \Pi \, S$ (S = solubility of drug, c taken as S)

Factors affecting drug release rate

Solubility:

APIs for **osmotic** delivery should have water solubility in the desired range to get optimize drug release. However, by modulating the solubility of these drugs within the core, effective release patterns may be obtained for the drugs, which might otherwise appear to be poor candidate for **osmotic** delivery.

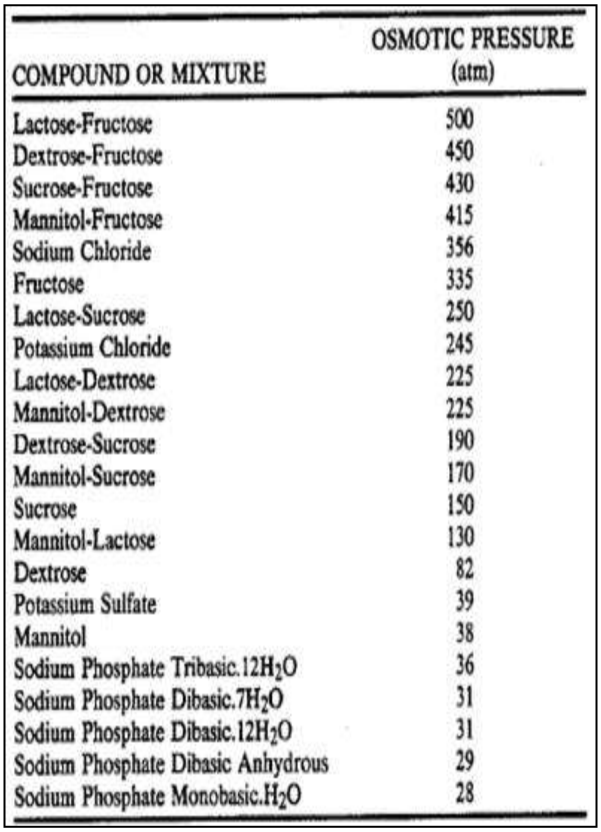
Solubility-modifying approaches:

- Use of swellable polymers: vinyl acetate copolymer, polyethylene oxide have uniform swelling rate which causes drug release at constant rate.
- Use of wicking agents: These agents may enhance the surface area of drug with the incoming aqueous fluids. e.g. colloidal silicon dioxide, sodium lauryl sulfate, etc. **Ensotrol**® technology uses the same principle to deliver drugs via **osmotic** mechanism.
- Use of effervescent mixtures: Mixture of citric acid and sodium bicarbonate which creates pressures in the **osmotic** system and ultimately controls the release rate.
- Use of cyclodextrin derivatives: They are known to increase solubility of poorly soluble drugs. The same phenomenon can also be used for the **osmotic** systems.
- Use of alternative salt form: Change in salt form of may change solubility.
- Use of encapsulated excipients: Solubility modifier excipient used in form of mini-

tablet coated with rate controlling membrane.

- Resin Modulation approach: Ion-exchange resin methods are commonly used to modify the solubility of APIs. Some of the resins used in **osmotic** systems are Poly (4-vinyl pyridine), Pentaerythritol, citric and adipic acids.
- Use of crystal habit modifiers: Different crystal form of the drug may have different solubility, so the excipient which may change crystal habit of the drug can be used to modulate solubility.
- Co-compression of drug with excipients: Different excipients can be used to modulate the solubility of APIs with different mechanisms like saturation solubility, pH dependent solubility. Examples of such excipients are organic acids, buffering agent, etc.

Table 3



COMPOUND OR MIXTURE	OSMOTIC PRESSURE (atm)
Lactose-Fructose	500
Dextrose-Fructose	450
Sucrose-Fructose	430
Mannitol-Fructose	415
Sodium Chloride	356
Fructose	335
Lactose-Sucrose	250
Potassium Chloride	245
Lactose-Dextrose	225
Mannitol-Dextrose	225
Dextrose-Sucrose	190
Mannitol-Sucrose	170
Sucrose	150
Mannitol-Lactose	130
Dextrose	82
Potassium Sulfate	39
Mannitol	38
Sodium Phosphate Tribasic.$12H_2O$	36
Sodium Phosphate Dibasic.$7H_2O$	31
Sodium Phosphate Dibasic.$12H_2O$	31
Sodium Phosphate Dibasic Anhydrous	29
Sodium Phosphate Monobasic.H_2O	28

Size of delivery orifice*:*

To achieve an optimal zero order delivery profile, the cross sectional area of the orifice must be smaller than a maximum size to minimize drug delivery by diffusion through the orifice. Furthermore, the area must be sufficiently large, above a minimum size to minimize hydrostatic pressure build up in the system. The typical orifice size in **osmotic** pumps ranges from 600µ to 1 mm.

Methods to create a delivery orifice in the **osmotic** tablet coating are:

- Mechanical drill
- Laser drill: This technology is well established for producing sub-millimeter size hole in tablets. Normally, CO_2 laser beam (with output wavelength of 10.6µ) is used for drilling purpose, which offers excellent reliability characteristics at low costs.
- Indentation that is not covered during the coating process: Indentation is made in core tablets by using modified punches having needle on upper punch. This indentation is not covered during coating process which acts as a path for drug release in **osmotic** system.
- Use of leachable substances in the semipermeable coating : e.g. controlled porosity osmotic pump

Basic components of Osmotic systems Drug:

Drugs which have short biological half-life and which are used for prolonged treatment are ideal candidate for **osmotic** systems. Various drug candidates such as Diltiazem HCl, Carbamazepine, Metoprolol, Oxprenolol, Nifedipine, Glipizide, etc are formulated as osmotic delivery.

Osmotic agent:

Osmotic components usually are ionic compounds consisting of either inorganic salts or hydrophilic polymers. Some of the **osmotic** agents that can be used for such systems are classified below. Different type of osmogents can be used for such systems are categorized as water-soluble salts of inorganic acids like magnesium chloride or sulfate; lithium, sodium, or potassium chloride; sodium or potassium hydrogen phosphate; water-soluble salts of organic acids like sodium and potassium acetate, magnesium succinate, sodium benzoate, sodium citrate, sodium ascorbate; Carbohydrates like mannose, sucrose, maltose lactose; water-soluble amino acids and organic polymeric osmogents, etc.

Semipermeable membrane:

An important part of the **osmotic** drug delivery system is the SPM housing. Therefore, the polymeric membrane selection is key to **osmotic** delivery formulation. The membrane must possess certain performance criteria such as:

- Sufficient wet strength and water permeability
- Should be biocompatible
- Rigid and non-swelling
- Should be sufficient thick to withstand the pressure within the device.

Any polymer that is permeable to water but impermeable to solute can be used as a coating material in **osmotic** devices. e.g. Cellulose esters like cellulose acetate, cellulose acetate butyrate, cellulose triacetate and ethyl cellulose and Eudragits.

Plasticizers:

Different types and amount of plasticizers used in coating membrane also have a significant importance in the formulation of **osmotic** systems. They can change visco-elastic behavior of polymers and these changes may affect the permeability of the polymeric films. Some of the plasticizers used are as below:

- Polyethylene glycols
- Ethylene glycol monoacetate; and diacetate- for low permeability
- Tri ethyl citrate
- Diethyl tartarate or Diacetin- for more permeable films

Table 4: Marketed products

Elementary osmotic pump

Brand Name	**API**
Efidac 24®	Chlorpheniramine
Acutrim ®	Phenylpropanolamine
Sudafed 24®	Pseudoephedrine
Volmax ®	Albuterol
Minipress XL®	Prazocine
Push-pull osmotic systems	
Ditropan XL ®	Oxybutynin chloride
Procardia XL®	Nifedipine
Glucotrol ®	Glipizide

Multiparticulate Drug Delivery System: (Dey NS 2008)

Introduction:

Multi-particulate drug delivery systems are mainly oral dosage forms consisting of a multiplicity of small discrete units, each exhibiting some desired characteristics. In these systems, the dosage of the drug substances is divided on a plurality of subunit, typically consisting of thousands of spherical particles with diameter of 0.05-2.00mm. Thus Multiparticulate dosage forms are pharmaceutical formulations in which the active substance is present as a number of small independent subunits. To deliver the recommended total dose, these subunits are filled into a sachet and encapsulated or compressed into a tablet. Multiparticulates are discrete particles that make up a multiple unit system. They provide many advantages over single-unit systems because of their small size. Multiparticulates are less dependent on gastric empty ting, resulting in less inter and intra-subject variability in gastrointestinal transit time. They are also better distributed and less likely to cause local irritation.

Mechanism of drug release from Multi-particulates (Dey NS 2008)

The mechanism of drug release from multiparticulates can be occurring in the following ways:

Diffusion

On contact with aqueous fluids in the gastrointestinal tract (GIT), water diffuses into the interior of the particle. Drug dissolution occurs and the drug solutions diffuse across the release coat to the exterior.

Erosion

Some coatings can be designed to erode gradually with time, thereby releasing the drug contained within the particle.

Osmosis

In allowing water to enter under the right circumstances, an osmotic pressure can be built up within the interior of the particle. The drug is forced out of the particle into the exterior through the coating.

Design of Multiparticulate drug delivery systems

(Dey NS 2008)

The purpose of designing Multiparticulate dosage form is to develop a reliable formulation that has all the advantages of a single unit formulations and yet devoid of the danger of alteration in drug release profile and formulation behavior due to unit to unit variation, change in gastro –luminal pH and enzyme population. A generally accepted view is that Multiparticulate systems perform better in vivo than single unit system, as they spread out through the length of the intestine cause less irritation, enjoy a slower transit through the colon and give a more reproducible drug release. As in the case of single unit dosage forms, for the purpose of the designing Multiparticulate colon specific drug delivery system, the presence of specific bacterial populations in the colon and increasing pH gradient have been extensively explored as triggering mechanism in order to initiate colon specific drug release.

Multiparticulate crystalline drug compositions

A Multiparticulate for controlled release of a drug comprises a crystalline drug, a glycerides having at least one alkylate substituent of not less than 16 carbon atoms, and a poloxamer, wherein at least 70 wt % of the drug in the Multiparticulate is crystalline. The Multiparticulate comprises crystalline drug particles embedded in the glycerides/ poloxamer mixture. The poloxamer 16 is substantially homogeneously distributed throughout the glycerides 14 and is present as a separate phase from the glycerides 14.

Multiparticulates as NDDS

Incorporating an existing medicine into a novel drug delivery system (NDDS) can significantly improve its performance in terms of efficacy, safety and improved patient compliance. In the form of a NDDS, an existing drug molecule can get new life, thereby increasing its market value and competitiveness and even extending patent life.

Intestinal Protective Drug Absorption System

Intestinal protective drug absorption system (IPDAS) (Figure 8) is a Multiparticulate tablet technology that has been developed to enhance the gastric tolerability of potentially irritant or ulcerogenic drugs such as the NSAIDs. It consists of high density controlled release beads that are compressed into a tablet form. The beads may be manufactured by techniques such as extrusion spheronization and controlled release can be achieved with the use of different polymer systems to coat the resultant beads. Alternatively, the drug can also be coated into on an inert carrier such as non-pareil seeds to produce instant release multiparticulates. Controlled release can be achieved by the formation of a polymeric membrane onto these instant release multparticulates. Once an IPDAS tablet is ingested, it rapidly disintegrates and disperses beads containing the drug in the stomach which subsequently pass into the duodenum and along the gastrointestinal tract in a controlled and gradual manner, independent of the feeding state. Release of active ingredient from the multiparticulates occurs through a process of diffusion either through the polymeric membrane and /or the micro matrix of the polymer/active ingredient formed in the extruded/spheronized multiparticulates. The intestinal protection of IPDAS is by virtue of the Multiparticulate nature of the formulation which ensures wide dispersion of irritant drug throughout the gastrointestinal tract. Naprelan®, which is marketed in the United States, employs IPDAS technology. This innovative formulation of naproxen sodium is a unique controlled release formulation indicated both for acute and chronic pain.

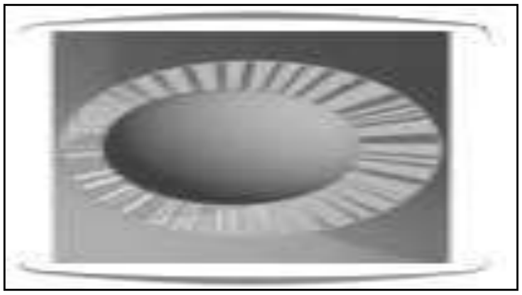


Fig 8: Intestinal Protective Drug Absorption System

Spheroidal oral drug absorption systems

Spheroidal Oral Drug Absorption System (SODAS) (Figure 9) is a Multiparticulate technology that enables the production of customized dosage forms and responds directly to individual drug candidate needs. It can provide a number of tailored drugs release profiles including immediate release of drug followed by sustained release to give rise to a fast onset of action which is maintained for 24 hours. Alternatively, the opposite scenario can be achieved where drug release is delayed for a number of hours.

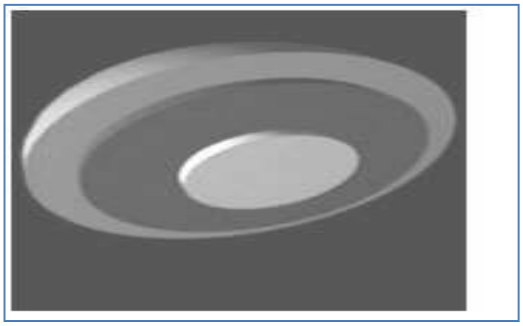


Fig 9: Spheroidal Oral Drug Absorption System

Programmable Oral Drug Absorption System

Programmable Oral Drug Absorption System (PRODAS) (Figure10) is presented as a number of mini tablets contained in hard gelatin capsule. It thus combines the benefits of tabletting technology within a capsule. It is possible to incorporate many different minitablets, each one formulated individually and programmed to release drug at different sites within the GIT. These combinations may include immediate release, delayed release, and/or controlled release mini tablets. It is also possible to incorporate mini tablets of different sizes so that high drug loading is possible. Their size ranges usually from 1.5 – 4 mm in diameter .

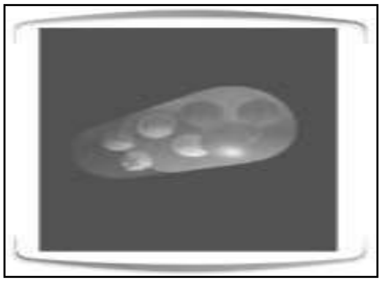


Fig 10: Programmable Oral Drug Absorption System

Minitabs

The Eurand MINITABS technology (Figure 12) is unique in that it offers the advantages of a tablet combined with those of a Multiparticulate drug form. Eurand MINITABS are tiny (2mm x 2mm) tablets containing gel forming excipients that control drug release rate. Additional membranes may be added to further control release rate. The small size of Eurand minitabs means that they can be filled into capsules as a final dosage form. As a result, combination products can be developed to allow for two or more release profiles within a single capsule. Eurand minitabs offer high drug loading, the ability to fine tune release rates for targeted delivery and content uniformity for more accurate dosing. Eurand Minitabs offer high drug loading, a wide range of release rate designs, and fine tuning of these release rates. The capsules can be opened and the contents used as a "sprinkle" formulation.

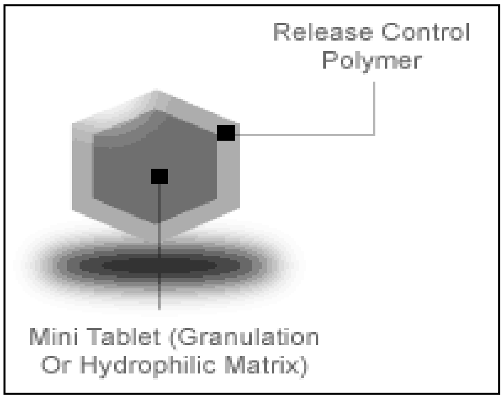


Fig 11: Minitab

Diffucaps

In this Multiparticulate system, drug profiles are created by layering an active drug onto a neutral core such as sugar spheres, crystals or granules followed by the application of a rate-controlling, functional membrane (Figure 12). The coating materials can be water soluble, pH dependent or independent or water insoluble depending on the individual needs of compound. The resultant beads are small in size approximately 1mm or less in diameter. By incorporating beads of differing drug release profiles into hard gelatin capsules, combination release profiles can be achieved. It is possible to customize any combination of sustained release, pulsatile release and immediate release profiles depending on the specific needs of the product.

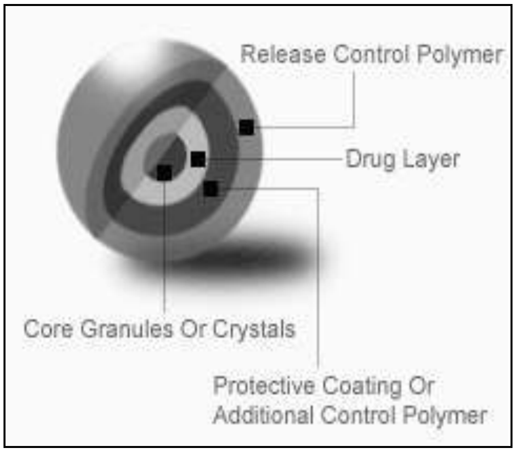


Fig 12: Diffucaps

The drug layering process can be conducted either from aqueous or solvent based drug solutions. Eurand has also developed a formulation technology that combines the customized drug release offered by Diffucaps with technologies that enhance the solubility of insoluble drugs in the gastrointestinal tract. Eurand is using this technology to provide a degree of delivery control that goes beyond that of single technology systems. Diffucaps beads are small in size, approximately 1mm in diameter, and are filled into a capsule to create the final dosage form. Beads of differing drug release profiles can be easily combined in a single capsule providing high levels of control over release profiles. Diffucaps beads of different drugs can be combined to make convenient single dose units for combination therapies.

Stabilized Pellet Delivery System

Stabilized pellet delivery system technology uses functional polymers or a combination of functional polymers and specific additives, such as composite polymeric materials to deliver a drug to a site of optimal absorption along the intestinal tract. The active drug is incorporated in Multiparticulate dosage forms such as DIFFUCAPS or Eurand MINITABS, which are then subsequently coated with pH dependent/independent polymeric membranes that will deliver the drug to the desired site. These are then filled into hard gelatin capsules. This technology is designed specifically for unstable drugs and incorporates a pellet core of drug and protective polymer outer layer(s).

Pelletized Delivery System

Pelletized Delivery System (PDS) is a sustained release system using pellets or beads manufactured using marumerization/ spheronization/pelletization techniques or by layering powders or solutions on nonpareil seeds. Release modulating polymers are sprayed on the beads using various coating techniques. The coated beads are filled in to hard gelatin capsules. Drug release occurs by diffusion associated with bioerosion or by osmosis via the surface membrane. The release mechanism can be pH-activated or pH-independent. The beads can be formulated to produce first order or zero order release.

Pelletised tablet

Pelletised tablet (Peltab®) system utilizes polymer-coated drug pellets or drug crystals, which are compressed into tablets. In order to provide a \controlled release, a water insoluble polymer is used to coat discrete drug pellets or crystals, which then can resist the action of fluids in the GIT. This technology incorporates a strong polymer coating enabling the coated pellets to be compressed into tablets without significant breakage.

Multiparticle Drug Dispersing Shuttle

Multiparticle drug dispersing shuttle (Multipart®) consists of a tablet carrier for the delivery of controlled release beads or pellets through the GIT which preserves the integrity and release properties of the beads. The distribution of the beads is triggered by the disintegration of the tablet carrier in the stomach. Drug release from the beads is triggered by super disintegration of the tablets. It can be pH-activated or pH-independent and can occur by disintegration or osmosis. The beads can be formulated to produce first or zero order release.

Macrocap®

Macrocap® consists of immediate release beads made by extrusion/ spheronization/ pelletization techniques or by layering powders or solutions on nonpareil seeds. Release modulating polymers are sprayed on the beads using various coating techniques. The coated beads are filled in hard gelatin capsules. Drug release occurs by diffusion associated with bioerosion or by osmosis via the surface membrane. The release mechanism can be pH-activated or pH independent. The beads can be formulated to produce first or zero order release.

Orbexa®

Orbexa® technology is a Multiparticulate system that enables high drug loading and is suitable for products that require granulation. This technology produces beads that are of controlled size and density using granulation, extrusion and spheronization techniques. This process is unique in that it allows for higher drug loading than other systems, is flexible and is suitable for use with sensitive materials such as enzymes.

KV/24

KV/24 is a patented, Multiparticulate drug delivery technology that encapsulates one or more drug compounds to achieve release in a pre-determined fashion over a 24-hour period after oral administration. KV/24 technology is based upon coating a neutral core (nonpareil bead) with a drug substance, then sequentially coating with one or more polymers to achieve a once-a day release profile. The drug can either be combined with the neutral core or incorporated into the coating process.

Flash tab

Flash tab technology is a fast dissolving/disintegrating oral tablet formulation. It is a combination of taste masked Multiparticulate active drug substances with specific excipients compressed into tablets. A disintegrating agent and a swelling agent are used in Combination with coated drug particles in this formulation to produce a tablet that disintegrates in the mouth in less than one minute. These oro-dispersible tablets disperse rapidly before the patient swallow them.

InnoHerb

This technology is used coating the pellets inside of the capsule, InnoHerb Phytogranules. The Multiparticulate is made up of many micropellets or small beads containing active herbal compounds. The special coating for each plant extract contains top quality standardized dried extracts which assures efficacy and safety of the semipermeable membrane, improves stability, mask taste/smell and affords gastro-protection as well as promote controlled release of actives, optimal availability and better absorption.

Layering process for Multiparticulate dosage form

Layering processes involve loading solid inert cores with drugs and/or excipients. Inert cores, placed in a suitable vessel such as a coating pan or a fluid bed, may be layered according to different methods. Some methods consist of spraying onto the cores a solution/suspension containing both drug and binding agent. Others are based on layering the drug directly in powdery form where drug loading occurs by gravity and adhesion is ensured by a liquid binder sprayed onto the cores. The layering process is particularly suitable for production of small drug loaded units, multiples of which are placed into capsules for patient delivery. In the case of spherical inert cores such as non-perils, the layering techniques from solution/suspensions produce homogeneous drug loaded particles, which retain an approximately spherical shape. They are therefore particularly suitable for successively film coating to build up the particle with the aim of providing a desired drug release profile.

Delayed release oral polypeptides

In one embodiment, the composition further includes an inert core. The inert core can be, e.g., a pellet, sphere or bead made up of sugar, starch, microcrystalline cellulose or any other pharmaceutically acceptable inert excipient. A preferred inert core is a carbohydrate, such as a monosaccharide, disaccharide, or polysaccharide, i.e., a polymer including three or more sugar molecules. An example of a suitable carbohydrate is sucrose. In some embodients, the sucrose is present in the composition at a concentration of 60-75%.

When the bioactive polypeptide is IL-11, the IL-11 layer is preferentially provided with a stabilizer such as methionine, glycine, polysorbate 80 and phosphate buffer, and/or a pharmaceutically acceptable binder, such as hydroxypropyl methylcellulose, povidone or hydroxypropyl ‘cellulose. The composition can additionally include one or more pharmaceutical excipients. Such pharmaceutical excipients include, e.g., binders, disintegrants, fillers, plasticizers, lubricants, glidants, coatings and suspending/ dispersing agents. In some embodiments, the composition is provided as a Multiparticulate system that includes a plurality of enteric coated, IL-11 layered pellets in a capsule dosage form. The enteric coated IL-11 pellets include an inert core, such as a carbohydrate sphere, a layer of IL-11 and an enteric coat. The enteric coat can include, e.g., a pH dependent polymer, a plasticizer, and an antisticking agent/glidant. Preferred polymers include e.g., methacrylic acid copolymer, cellulose acetate phthalate, hydroxyl propyl methylcellulose phthalate, polyvinyl acetate phthalate, shellac, hydroxylpropyl methylcellulose acetate succinate and carboxymethylcellulose. Preferably, an inert seal coat is present in the composition as a barrier between the IL-11 layer and enteric coat. The inert seal coat can be e.g. hydroxyl propyl methyl cellulose, povidone, hydroxylpropyl cellulose or another pharmaceutically acceptable binder. Suitable sustained release polymers include, e.g., amino methacrylate copolymers (Eudragit RL, Eudragit RS), ethylcellulose or hydroxypropyl methylcellulose. In some embodiments, the methacrylic acid copolymer is a pH dependent anionic polymer solubilizing above pH 5.5. The methacrylic acid copolymer can be provided as a dispersion and be present in the composition at a concentration of 10-20% wt/wt. A preferred methacrylic acid copolymer Eudragit®.

Multiparticulate mucoadhesive formulations:

In a preferred embodiment, illustrated by way of example, there is provided a supply of gas developing components as well as a number of film-like, stacked individual particles which consist of a mucoadhesive, active substancecontaining layer and of a backing layer controlling the direction of active substance release, these components being located within a polymer enclosure which is resistant to gastric juice but permeable to intestinal juice. Here, the active substance may be present embedded in the film-like component. The process for the production of a gastric juice-resistant device, consisting of at least one active substance in the form of a Multiparticulate preparation with mucoadhesive properties, and of a blowing agent which on contact with liquid produces gas individual particles being enclosed by a gastric juice-resistant, intestinal juice-soluble polymer enclosure, is as follows:

(a) transfer of apolymer material in web form to a molding board provided with bores, and applying a vacuum to form the compartments of the polymer enclosure
(b) alternately filling-in the active substance-containing preparation and the blowing agent-containing preparation
(c) superposing a second polymer web, and closing the compartments by sealing with application of heat and pressure
(d) Separating the individual devices by punching or cutting.

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GASTRORETENTIVE DRUG DELIVERY SYSTEM

Basrur Pooja, Hastak Vishakha

Introduction:

Oral delivery of drugs is the most preferable route of drug delivery due to the ease of administration, patient compliance and flexibility in formulation. (Sanjay 2003) The oral route is considered as the most promising route of drug delivery. Conventional drug delivery system achieves as well as maintains the drug concentration within the therapeutically effective range needed for treatment. (Praveen 2010) Oral controlled drug is precluded by several physiological difficulties. Delivery system (DDS) should be primarily aimed at achieving more predictable and increased bioavailability of drugs. The relatively brief GET in humans which normally averages 2–3 h through the major absorption zone (stomach or upper part of the intestine), can result in incomplete drug release from the DDS leading to diminished efficacy of the administered dose. The intimate contact of the DDS with the absorbing membrane has the potential to maximize drug absorption and the rate of drug absorption. These considerations have led to the development of oral controlled- release (CR) dosage forms possessing gastric retention capabilities. (Brahma N 2000).

Gastro retentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment. It has applications also for local drug delivery to the stomach and proximal small intestines. Gastro retention helps to provide better availability of new products with new therapeutic possibilities and substantial benefits for patients. (Amit K 2011)

Advantages of Gastroretentive Drug Delivery Systems:

1. Drugs acting locally in the stomach, drugs which degrade in the colon and those having rapid absorption through GIT are formulated by use of this system. This site-specific drug delivery reduces undesirable side effects. It improves patient compliance. (Praveen 2010)
2. The bioavailability of therapeutic agents can be significantly enhanced especially for those which get metabolized in the upper GIT by this gastroretentive drug delivery approach.
3. For drugs with relatively short half life, sustained release may enable reduced frequency of dosing with improved patient compliance.
4. These overcome the adversities of the gastric retention time (GRT) as well as the gastric emptying time (GET). They are expected to remain buoyant on the gastric fluid without affecting the intrinsic rate of employing because their bulk density is lower than that of the gastric fluids.
5. Gastroretentive dosage forms minimize the fluctuation of drug concentrations and effects. This feature is of special importance for drug with a narrow therapeutic index.
6. Reduction of fluctuation in drug concentration makes it possible to obtain improved selectivity in receptor activation. (Amit N 2010)

Drugs suitable for GRDDS:

1) Drugs those are locally active in the stomach e.g. misroprostol, antacids etc.
2) Drugs that have narrow absorption window in gastrointestinal tract (GIT) e.g. L-DOPA, Para amino benzoic acid, furosemide, riboflavin etc.
3) Drugs those are unstable in the intestinal or colonic environment e.g. captopril, ranitidine HCl, metronidazole.
4) Drugs that disturb normal colonic microbes e.g. antibiotics against Helicobacter pylori.
5) Drugs that exhibit low solubility at high pH values e.g. diazepam, chlordiazepoxide, verapamil HCl.
6) Drugs primarily absorbed from stomach and upper part of Gastro-intestinal tract, e.g., calcium supplements, cinnarazine

Drugs unsuitable for GRDDS:

1) Drugs that have very limited acid solubility e.g. phenytoin.
2) Drugs that suffer instability in the gastric environment e.g. erythromycin.
3) Drugs intended for selective release in the colon e.g. 5-amino salicylic acid and corticosteroids.

Factors affecting gastric retention:

Density of dosage forms:

FDDS are retained in the stomach for a prolonged period of time by virtue of their floating properties, pylorus which can be acquired by several means. The density of a dosage form affects the gastric emptying rate and determines the location of the system in the stomach. In order for a HBS dosage form to float in the stomach density of the dosage form should be less than the gastric contents. A density of less than 1.0 g/ml has been reported in the literature. The buoyant capabilities are better represented and monitored by resultant-weight measurements and swelling experiments. This is because the magnitude of floating boundary may vary as a function of time and usually decreases after immersion of the dosage form into the fluid as a result of the development of its hydrodynamic equilibrium. Dosage forms having a density lower than the gastric contents can float to the surface, while high density systems sink to bottom of the stomach. Both positions may isolate the dosage system from the pylorus.

Shape and size of the dosage form:

Small-size tablets are emptied from the stomach during the digestive phase, while larger-size are expelled. The effect of size on the gastric retention time of floating and non-floating units can be studied using γ-scintigraphy. The mean gastric residence times of non-floating dosage forms are highly variable and greatly dependent on their size. The Floating units with a smaller diameter had longer GRTs compared to Non-Floating units. The Floating units remain buoyant on gastric contents, are protected against gastric emptying during digestive phases. On the other hand, Non-Floating units lie in the antrum region and are propelled during the digestive process by peristalsis. Ring-shaped and tetrahedron-shaped devices have a better gastric residence time as compared with other shapes.

Food intake and its nature:

Food intake, viscosity and volume of food, caloric value and frequency of feeding have a profound effect on the gastric retention of dosage forms. The presence or absence of food in the gastrointestinal tract (GIT) influences the gastric retention time (GRT) of the dosage form. Usually the presence of food in the gastrointestinal tract (GIT) improves the gastric retention time (GRT) of the dosage form and thus, the drugs absorption increases by allowing its stay at the absorption site for a longer period. Increase in acidity and caloric value shows decreased gastric emptying time (GET), which can improve the gastric retention of dosage forms. It is reported that in the fed state after a single meal, all the floating units had a floating time (FT) of about 5 h and a GRT prolonged by about 2 h over the control. Food intake is the main determinant of gastric emptying, while specific gravity has only a minor effect on the emptying process. The presence of food, rather than the buoyancy, is the most important factor affecting GRT and floating does not invariably increase GRT. In fact, studies have shown that the GET for both Floating (F) and non-floating (NF) single units are shorter in fasted subjects (less than 2 h), but are significantly prolonged after a meal (around 4 h). (Brahma N 2000)

Effect of gender, posture and age:

Females generally have slower gastric emptying rates than male. The effect of posture does not have any significant difference in the mean gastric retention time (GRT) for individuals in upright, ambulatory and supine state. (Amit N 2010)

Disease states:
Diseases like gastroenteritis, gastric ulcer, pyloric stenosis, diabetes and hypothyroidism retard gastric emptying, while partial or total gastrectomy, duodenal ulcer and hyperthyroidism promote it.

Concomitant drug administration:
Certain drugs retard gastric emptying e.g. poorly soluble antacids (aluminum hydroxide), anticholinergics (atropine, propantheline), narcotic analgesics (morphine). Drugs such as tricyclic antidepressants (imipramine, amitriptyline), metoclopramide, domperidone, cisapride stimulate gastric emptying.

Single or multiple unit formulation:
Multiple unit formulations show a more predictable release profile and insignificant impairing of performance due to failure of units, allow co-administration of units with different release profiles or containing incompatible substances and permit a larger margin of safety against dosage form failure compared with single-unit dosage forms. (Abhishek S 2012)

Gastrointestinal tract & its role in gastro retention:

The oral route remains the preferred route for the administration of therapeutic agents because the low cost of therapy and ease of administration lead to high levels of patient compliance. The control of gastrointestinal transit of orally administered dosage forms using gastroretentive drug delivery systems (GRDDS) can improve the bioavailability of drugs that exhibit site-specific absorption. Prolonged gastric retention can be achieved by using floating, swelling, bioadhesive, or high-density systems. Conventional oral dosage forms provide a specific drug concentration in systemic circulation without offering any control over drug delivery. Controlled-release drug delivery systems (CRDDS) provide drug release at a predetermined, predictable, and controlled rate. An important requisite for the successful performance of oral CRDDS is that the drug should have good absorption throughout the gastrointestinal tract (GIT), preferably by passive diffusion, to ensure continuous absorption of the released drug. The average time required for a dosage unit to traverse the GIT is 3–4 h, although slight variations exist among various dosage forms. (Arvind 2003)

The stomach is J-shaped organ located in the upper left hand portion of the abdomen, just below the diaphragm. It occupies a portion of the epigastric and left hydrochondriac region. The main function of the stomach is to store the food temporarily, grind it and then release it slowly into the duodenum. Due to its small surface area very little absorption takes place from the stomach. It provides barrier to the delivery of drugs to small intestine.
The stomach is divided into three anatomical regions:
i) Fundus
ii) Body
iii) Pylorus (or antrum).
The proximal stomach consisted of Fundus and body, which serves as a reservoir for ingested materials, whereas the distal region (pylorus) is the major site of mixing motions, acting as a pump to propel gastric contents for gastric emptying. (Amit K 2011)

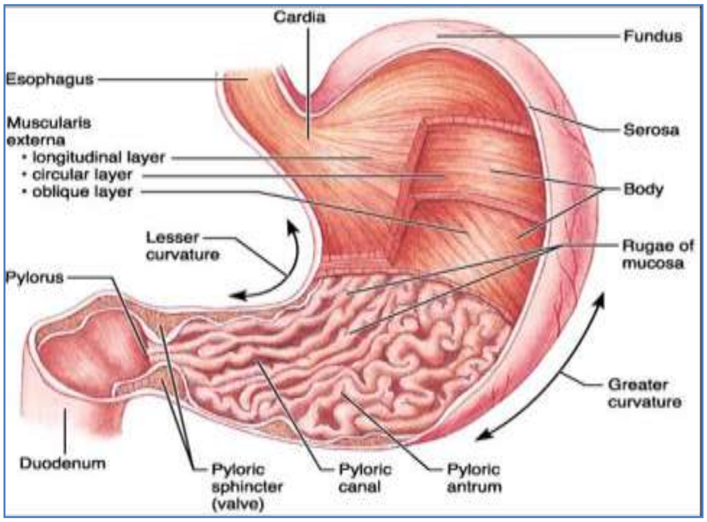

Figure 1:- Anatomy of Stomach

All drug candidates are not absorbed uniformly throughout the GIT. An absorption window exists because of physiological, physicochemical, or biochemical factors. The pH-dependent solubility and stability level of a drug plays an important role in its absorption. A drug must be in a solubilized and stable form to successfully cross the biological membrane, and it will experience a pH range from 1 to 8 as it travels through the GIT. Most drugs are absorbed by passive diffusion of the un-ionized form, the extent of ionization at various pH levels can lead to nonuniform absorption or an absorption window. The presence of certain enzymes in a particular region of the GIT also can lead to regional variability in the absorption of drugs that are substrates of those enzymes. Drugs absorbed by active and facilitated transport mechanisms show higher regional specificity because of the prevalence of these mechanisms in only certain regions of the GIT. Intestinal metabolic enzymes primarily cytochrome P450 (CYP3A)—are abundantly present in the intestinal epithelium. Nonuniform distribution of CYP3A causes regional variability in the absorption of drugs that are substrates of that enzyme.

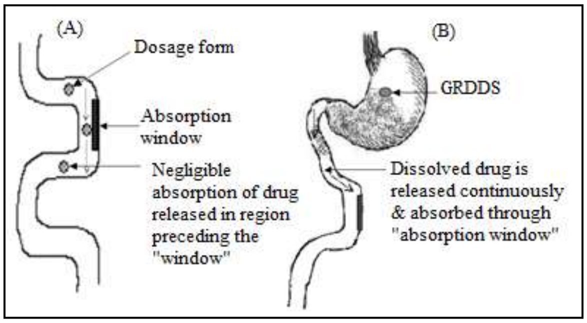
(A)
Dosage form
Absorption window
Negligible absorption of drug released in region preceding the "window"
(B)
GRDDS
Dissolved drug is released continuously & absorbed through "absorption window"

Figure 2:- Drug absorption in the case of (A) conventional dosage forms and (B) gastroretentive drug delivery systems.

GRDDS can improve the controlled delivery of drugs for a prolonged period of time by continuously releasing the drug before it reaches its absorption site thus ensuring its optimal bioavailability. The bioavailability of orally administered drugs will vary depending on the state of feeding. Gastric emptying occurs both in fasting as well as fed states.

The fasted state is associated with various cyclic events, commonly referred to as the migrating motor complex (MMC), which regulates GI motility patterns. The MMC is organized into alternating cycles of activity and quiescence and can be subdivided into:

Phase I- Basal phase, lasts from 30 to 60 min and is characterized by a lack of secretory, electrical, and contractile activity.

Phase II- Preburst phase, exhibits intermittent action for 20–40 min, during which contractile motions increase in frequency and size.

Phase III- Burst phase, characterized by intense, large, and regular contractions, termed housekeeper waves that sweep off undigested food and last 10–20 min.

Phase IV is the transition period of 0 -5 min between Phases III and I. (Arvind 2003)

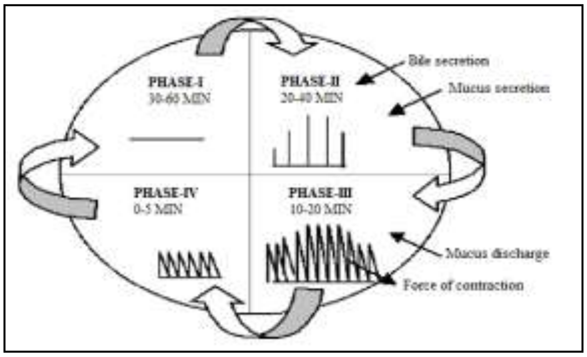

Figure 3: Motility patterns of the GIT in the fasted state.

After ingestion of mixed meal, the pattern of contractions change from fasted to fed state. Digestive motility pattern comprises of continuous contractions as in phase II of fasted state. These contractions result in reducing size of food particles, which are propelled towards the pylorus in a suspension form. During the fed state onset of MMC is delayed resulting in slowdown of gastric emptying rate. It can be concluded that feeding results in a lag time before onset of gastric emptying cycle. (Kumar 2011)

Classification of GRDDS:

Certain factors should be considered during development of gastroretentive dosage form. They include retention in the stomach according to the clinical demand, convenient intake, and ability to load substantial amounts of drugs with different physicochemical properties and release them in controlled manner, preferably in the stomach. Various approaches have been pursued to increase the retention of an oral dosage form in the stomach including floating systems, swelling and expanding systems, bioadhesive systems, modified-shape systems, super porous hydrogel, magnetic systems, high-density systems, and delayed gastric emptying devices.

A. FLOATING DRUG DELIVERY SYSTEM:

Floating systems are low-density systems that have sufficient buoyancy to float over the gastric contents and remain in the stomach for a prolonged period. While the system floats over the gastric contents, the drug is released slowly at the desired rate, which results in increased GRT and reduces fluctuation in plasma drug concentration. (Arvind 2003)
Floating drug delivery systems (FDDS) have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. (Sanjay 2003) Most of the floating systems are single-unit, such as the HBS and floating tablets. These systems are unreliable and irreproducible in prolonging residence time in the stomach when orally administered. On the other hand, multiple-unit dosage forms appear to be better suited since they are claimed to reduce the inter-subject variability in absorption and lower the probability of dose-dumping. (Brahma N 2000) Floating systems can be classified as effervescent and non-effervescent systems.

i. Non-effervescent Systems:

This system, after swallowing, swells via imbibing gastric fluid to an extent that it prevents its exit from the stomach. These systems may be referred to as the 'plug-type systems' since they have a tendency to remain lodged near the pyloric sphincter. The air trapped by the swollen polymer confers buoyancy to these dosage forms. (Sanjay 2003) They maintain a relative integrity of shape and a bulk density of less than unity within the outer gelatinous barrier. The gel structure acts as a reservoir for sustained drug release since the drug is slowly released by a controlled diffusion through the gelatinous barrier.
Commonly used excipients in non-effervescent FDDS are gel-forming or highly swellable cellulose type hydrocolloids, polysaccharides, and matrix forming polymers such as polycarbonate, polyacrylate, polymethacrylate and polystyrene.

- **Colloidal gel barrier system:**

These are designated as "Hydrodynamically balanced system". They contain drug with gel-forming hydrocolloids meant to remain buoyant on the stomach content. They prolong GRT and maximize the amount of drug that reaches its absorption sites in the solution form for ready absorption. These systems incorporate a high level of one or more gel-forming highly soluble cellulose type hydrocolloid, matrix-forming polymer such as polycarbophil on coming in contact with gastric fluid, the hydrocolloid in the system hydrates and forms a colloid gel barrier around its surface. The air trapped by the swollen polymer confers buoyancy to these dosage forms.

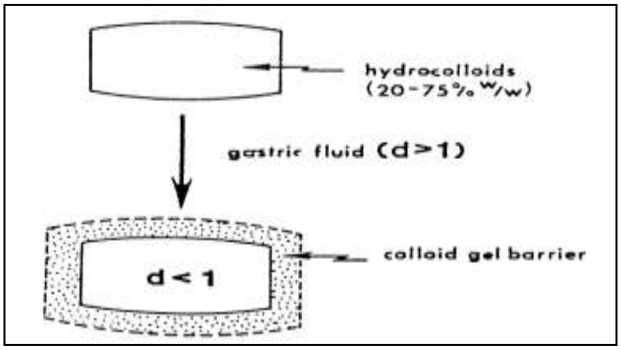

Figure 4: Intragastric floating tablet releasing drug via colloidal gel barrier

- **Microporous compartment system:**

Drug reservoir is encapsulated inside a Microporous compartment with pores along its top and bottom walls. The peripheral walls of the drug reservoir compartment are completely sealed to prevent any direct contact of gastric surface with the undissolved drug. In the stomach, the floatation chamber containing entrapped air causes the delivery system to float over the gastric content. Gastric fluid enters through the aperture, dissolves the drug and carries the dissolved drug for continuous transport across the intestine for absorption.

- **Alginate beads:**

Multi-unit floating dosage forms are developed from freeze-dried calcium alginate. Spherical beads of approximately 2.5 mm in diameter can be prepared by dropping sodium alginate solution into aqueous solution of calcium chloride, causing the precipitation of calcium alginate. The beads are then separated, snap-frozen in liquid nitrogen, and freeze-dried at -40ºC for 24 hours, leading to the formation of a porous system, which can maintain a floating force for over 12 hours. These floating beads give a prolonged residence time of more than 5.5 hours.

Evaluation:

Particle size and morphology of gel beads:

The mean diameters of dried beads are determined by optical microscopy. Morphological examination of the surface and internal structure of the dried beads was carried out using a scanning electron microscope. The samples are coated with gold to a thickness of about 30 nm in a vacuum evaporator. The internal structures of the beads were examined by cutting them in half with a steel blade.

Buoyancy of gel beads:

The gel bead samples are placed in the Erlenmeyer flask filled with 50 ml of simulated gastric fluid USP without pepsin (SGF) test solution. The flask is shaken in a shaking incubator. The shaking speed is 100 rpm and the temperature is maintained at 37 ◦C. Their buoyancy is observed for 24 h. The preparation is considered to have buoyancy in the test solution only when all of the gel beads floated in it. (Pornsak S 2005)

- **Hollow microspheres / Microballons:**

Hollow microspheres loaded with drug in their outer polymer shelf were prepared by a novel emulsion solvent diffusion method. The solution of the drug and an enteric acrylic polymer is poured into an agitated solution of Poly Vinyl Alcohol (PVA) that was thermally controlled at 40ºC. The gas phase is generated in the dispersed polymer droplet by the evaporation of dichloromethane formed and internal cavity in the microsphere of the polymer with drug. The Microballons floated continuously over the surface of an acidic dissolution media containing surfactant for more than 12 h. (Garg R 2008)

Evaluation:

Buoyancy:

Microballons are dispersed in solution containing Tween 20 (0.02 w/v %). Buoyancy is determined by the weight ratio of the floating particles to the sum of floating and sinking particles.

Apparent particle density:

Apparent particle density is determined by the projective image count method.

Drug release:

The drug release from Microballons is measured by performing dissolution test. (Yasunori S 2003)

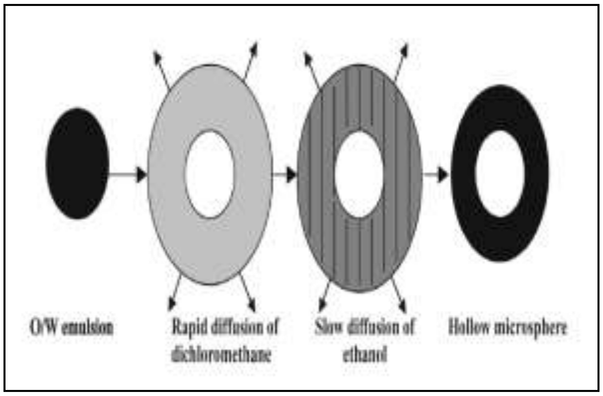

Figure 5: Formulation of Microballons

ii. **Effervescent Systems:**

These buoyant delivery systems utilize matrices prepared with swellable polymers such as polysaccharides, e.g., chitosan, and effervescent components, e.g., sodium bicarbonate and citric or tartaric acid or matrices containing chambers of liquid that gasify at body temperature. The matrices are fabricated so that upon arrival in the stomach, carbon dioxide is liberated by the acidity of the gastric contents and is entrapped in the jellified hydrocolloid. This produces an upward motion of the dosage form and maintains its buoyancy. A decrease in specific gravity causes the dosage form to float on the chyme. The carbon dioxide generating components may be intimately mixed within the tablet matrix. Gas can also be introduced into the floating chamber by the volatilization of an organic solvent (e.g., ether or cyclopentane). These devices contain a hollow deformable unit that converts from a collapsed to an expanded position and returns to the collapsed position after a predetermined amount of time to permit the spontaneous ejection of the inflatable system from the stomach

Carbonates, in addition to imparting buoyancy to these formulations, provide the initial alkaline microenvironment for polymers to gel. Moreover, the release of carbon dioxide helps to accelerate the hydration of the floating tablets which is essential for the formation of a bioadhesive hydrogel. This provides an additional mechanism ('bioadhesion") for retaining the dosage form in the stomach, apart from floatation.

a) Gas-generating Systems:

These are low density FDDS based on the formation of carbon-dioxide within the device following contact with body fluids. A single layer tablet or a Bilayer tablet may be compressed which contains the gas-generating mechanism in one hydrocolloid containing layer and the drug in the other layer formulated for a sustained release effect. The coating, is usually insoluble but permeable, allows permeation of water.

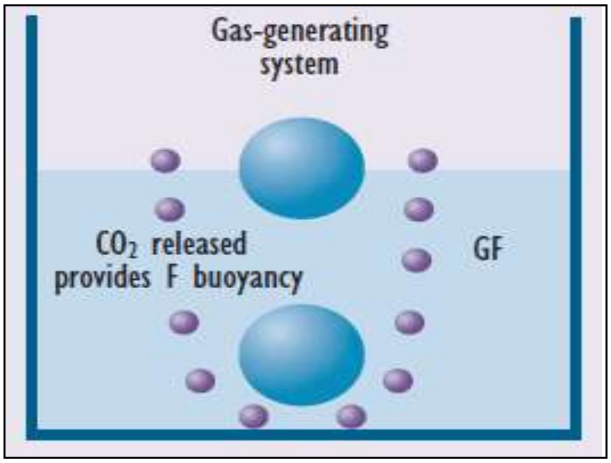

Figure 6: Gas-generating system

- **Intra Gastric Single Layer Floating Tablets or Hydrodynamically Balanced System (HBS):**

These are formulated by intimately mixing the CO2 generating agents and the drug within the matrix tablet. These have a bulk density lower than gastric fluids and therefore remain floating in the stomach unflattering the gastric emptying rate for a prolonged period. The drug is slowly released at a desired rate from the floating system and after the complete release the residual system is expelled from the stomach. This leads to an increase in the GRT and a better control over fluctuations in plasma drug concentration.

- **Intra Gastric Bilayer Floating Tablets:**

These are also compressed tablets contain two layers
i. Immediate release layer and
ii. Sustained release layer.

- **Multiple Unit type floating pills:**

These systems consist of sustained release pills as 'seeds' surrounded by double layers. The inner layer consists of effervescent agents while the outer layer is of swellable membrane layer. When the system is immersed in dissolution medium at body temp, it sinks at once and then forms swollen pills like balloons, which float as they have lower density. This lower density is due to generation and entrapment of carbon dioxide within the system.

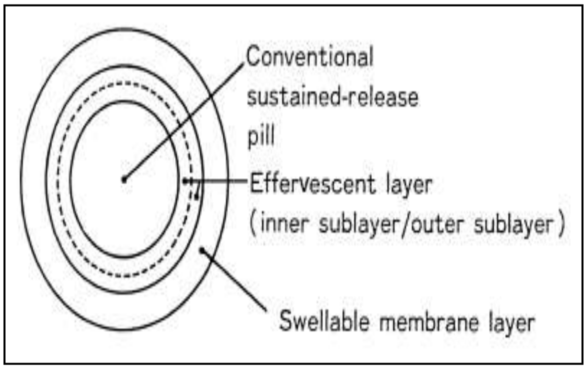
Conventional sustained-release pill
Effervescent layer (inner sublayer/outer sublayer)
Swellable membrane layer

Figure 7: A multiple-unit oral floating dosage system.

Evaluation of Multiple unit dosage form

Morphological and dimensional analysis: With the aid of scanning electron microscopy (SEM), the size can be measured using an optical microscope.

% yield of microspheres: This is calculated from weight of microspheres obtained ×100 total weight of drug and polymer.

Entrapment efficiency: The drug is extracted by a suitable method, analyzed and is calculated from:

$$\frac{\text{Practical amount of drug present}}{\text{Theoretical drug content}} \times 100$$

In vitro floating ability (Buoyancy %):

A known quantity of microspheres are spread over the surface of a USP (Type II) dissolution apparatus filled with 900 ml of 0.1 N HCl containing 0.002% v/v Tween 80 and agitated at 100 rpm for 12 hours. After 12 hours, the floating and settled layers are separated, dried in a dessicator and weighed. The buoyancy is calculated from the following formula.

Buoyancy (%) = $W_f / (W_f + W_s)$ X 100

Where W_f and W_s are the weights of floating and settled microspheres respectively.

Drug-excipient (DE) interactions:

This is done using FTIR. Appearance of a new peak, and/or disappearance of original drug or excipient peak indicate the DE interaction. Apart from the above mentioned evaluation parameters, granules (ex: Gelucire 43/01) are also evaluated for the effect of ageing with the help of Differential Scanning Calorimeter or Hot stage polarizing microscopy.

b) Volatile Liquid / Vacuum Containing Systems:

The device comprises of a hollow deformable unit that can be converted from a collapsed to an expanded position and returned to a collapsed position after an extended period of time. Housing is attached to the deformable unit and separated by an impermeable, pressure responsive movable bladder.

- **Intragastric Floating Gastrointestinal Drug Delivery System:**

These systems can be made to float in the stomach because of floatation chamber, which may be a vacuum or filled with air or a harmless gas, while drug reservoir is encapsulated inside a Microporous compartment.

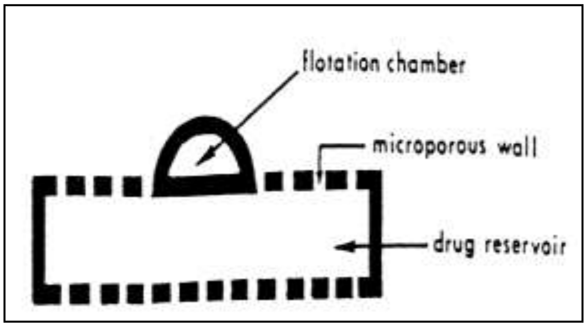

Figure 8: Intragastric floating drug delivery device

- **Inflatable Gastrointestinal Delivery Systems:**

In these systems an inflatable chamber is incorporated, which contains liquid ether that gasifies at body temperature to cause the chamber to inflate in the stomach. These systems are fabricated by loading the inflatable chamber with a drug reservoir, which can be a drug, impregnated polymeric matrix, then encapsulated in a gelatin capsule. After oral administration, the capsule dissolves to release the drug reservoir together with the inflatable chamber. The inflatable chamber automatically inflates and retains the drug reservoir compartment in the stomach. The drug continuously released from the reservoir into the gastric fluid.

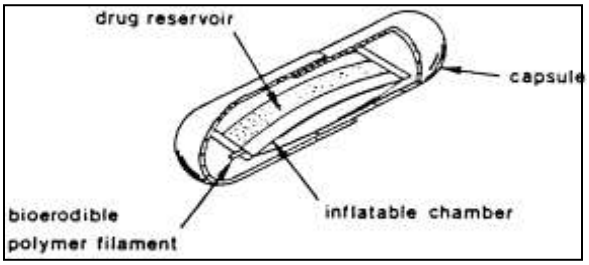

Figure 9: Gastro-inflatable drug delivery device

- **Intragastric Osmotically Controlled Drug Delivery System:**

It is comprised of an osmotic pressure controlled drug delivery device and an inflatable floating support in a biodegradable capsule. In the stomach, the capsule quickly disintegrates to release the drug from the delivery device. The inflatable support inside forms a deformable hollow polymeric bag that contains a liquid that gasifies at body temperature to inflate the bag. The osmotic pressure controlled drug delivery device consists of two components; drug reservoir compartment and an osmotically active compartment. The floating support is also made to contain a Bioerodable plug that erodes after a predetermined time to deflate the support. The deflated drug delivery system is then emptied from the stomach. Although this type of sophisticated dosage form might be used to administer a drug at a controlled rate for a prolonged period of time, it could not be recommended for smokers because of safety reasons. (Amit K 2011)

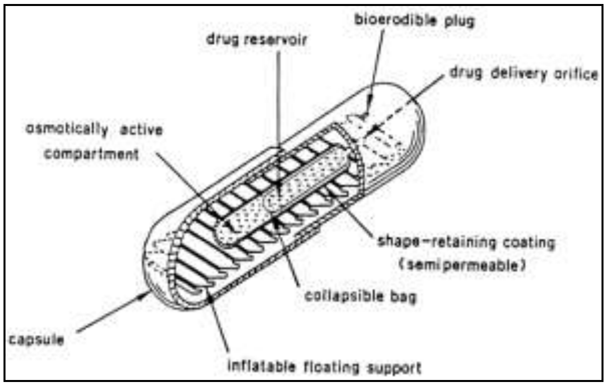
bioerodible plug
drug reservoir
drug delivery orifice
osmotically active compartment
shape-retaining coating (semipermeable)
collapsible bag
capsule
inflatable floating support

Figure 10: Intragastric osmotic controlled drug delivery system

iii. Raft forming system:

Raft forming systems have been used for the delivery of drugs for gastrointestinal infections and disorders. The mechanism involved in the raft formation includes the formation of viscous cohesive gel in contact with gastric fluids, wherein each portion of the liquid swells forming a continuous layer called a raft. This raft floats on gastric fluids because of low bulk density created by the formation of carbon dioxide. Usually, the system ingredients include gel forming agent and alkaline bicarbonates or carbonates responsible for the formation of carbon dioxide to make the system less dense and float on the gastric fluids. The raft floats on the gastric fluid and prevents the reflux of the gastric contents (i.e. gastric acid) into the esophagus by acting as a barrier between the stomach and esophagus. (Praveen 2010)

Evaluation of Floating tablets:

Evaluation of powder blend:

a) **Angle of repose:**

Angle of repose is defined as “the maximum angle possible between the surface of the pile of powder and the horizontal plane.” Lower the angle of repose, better the flow properties. The angle of repose may be calculated by measuring the height (h) of the pile and the radius of the base(r) with ruler.

$\tan \theta = h/r$

b) **Bulk density:**

Bulk density denotes the total density of the material. It includes the true volume of interparticle spaces and intraparticle pores. The packing of particles is mainly responsible for bulk .Bulk density is defined as:

$$\text{Bulk density} = \frac{\text{Weight of the powder}}{\text{Bulk volume of powder}}$$

c) **Percentage porosity:**

Whether the powder is porous or nonporous, the total porosity expression for the calculation remains the same. Porosity provides information about hardness, disintegration, total porosity etc.

$$\%\ \text{porosity},\ € = \frac{\text{void volume x100}}{\text{Bulk volume}}$$

$$\%\ \text{porosity},\ € = \frac{\text{(bulk volume-true volume) x100}}{\text{True density}}$$

Evaluation of floating tablets:

a) Floating lag time:

The buoyancy lag time is determined in the U.S.P. dissolution test apparatus II in an acid environment. The time interval between the introduction of the tablet into the dissolution medium and its buoyancy to the top of the dissolution medium is buoyancy lag time or floating lag time.

b) **In vitro drug release and duration of floating:**

This is determined by using USP II apparatus (paddle) stirring at a speed of 50 or 100 rpm at 37 ± 0.2 °c in simulated gastric fluid (pH 1.2 without pepsin). Aliquots of the samples are collected and analyzed for the drug content. The time (hrs) for which the tablets remain buoyant on the surface of the dissolution medium is the duration of floating and is visually observed.

c) Tablet swelling indices:

Tablets were weighed (W1) and placed in a glass beaker, containing 200 ml of 0.1 N HCl, maintained in a water bath at 37 ± 0.5° C. At regular time intervals, the tablets were removed and the excess surface liquid was carefully removed by a filter paper. The swollen tablets were then reweighed (W2). The swelling index (SI) was calculated using the formula:

$$\mathbf{SI = W_2 - W_1 / W_1}$$

d) In vivo evaluation for gastro-retention:

This is carried out by means of X-ray or Gamma scintigraphy monitoring of the dosage form transition in the GIT. X-Ray/Gamma Scintigraphy is a very popular evaluation parameter for floating dosage form nowadays. It helps to locate dosage form in the gastrointestinal tract (GIT), by which one can predict and correlate the gastric emptying time and the passage of dosage form in the GIT. Here the inclusion of a radio-opaque material into a solid dosage form enables it to be visualized by X-rays. Similarly, the inclusion of a γ-emitting radionuclide in a formulation allows indirect external observation using a γ-camera or scinti-scanner. In case of γ-scintigraphy, the γ-rays emitted by the radionuclide are focused on a camera, which helps to monitor the location of the dosage form in the GIT

e) **Hardness:**

The Monsanto hardness tester is used to determine the tablet hardness. The tablets are held between affixed and moving jaw. Scale is adjusted to zero; load is gradually increased until the tablet fractured. The value of the load at that point gives a measure of the hardness of the tablet which is expressed in kg/cm^2.

f) **Friability:**

Tablet strength is tested by Roche friabilator. Pre weighed tablets are allowed for 100 revolutions in 4 min and were dedusted. The percentage weight loss was calculated by reweighing the tablets. The % friability was then calculated by: -

$$F = (W_{initial}) - (W_{final}) \times 100 / (W_{initial})$$

g) **Weight variation:**

Randomly selected 20 tablets are weighed individually and together in a single pan balance. The average weight is noted and standard deviation is calculated. The tablet passes the test if not more than two tablets fall outside the percentage limit and none of the tablet differs by more than double percentage limit.

$$PD = (W_{average}) - (W_{initial}) / (W_{average}) \times 100$$

PD= percentage Deviation (Prakash R 2009)

B. EXPANDABLE SYSTEMS:

These GRDFs are easily swallowed and reach a significantly larger size in the stomach due to swelling or unfolding processes that prolong their GRT. After drug release, their dimensions are minimized with subsequent evacuation from the stomach. Gastroretentivity is enhanced by the combination of substantial dimensions with high rigidity of the dosage form to withstand the peristalsis. These systems are sometimes referred to as plug type systems because they tend to remain lodged at the pyloric sphincter.

- **Swelling GRDF's:**

These are comprised of an envelope from an elastic or non elastic non hydra -table polymeric membrane, which is drug and body fluid permeable. The envelope contains a drug reservoir and an expanding agent i.e. a swellable resin or hydrocolloid which causes expansion by osmotic pressure. Such devices of sizes larger than 1.531 cm were retained in the stomach for prolonged periods of time, typically than 12 h. The proposed mechanism of retention is not only due to large dimensions, but also by maintaining the stomach in the fed mode i.e. delaying the 'housekeeper wave' Administration of riboflavin-5-phosphate containing hydrogel to dogs maintained elevated blood concentrations for up to 54 h, thus yielding a 3.7-fold increase in bioavailability in comparison to oral bolus.

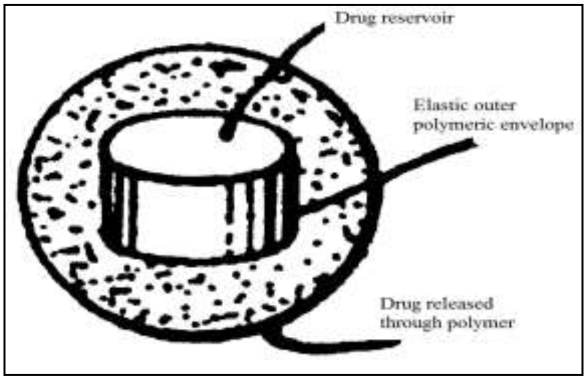
Drug reservoir
Elastic outer polymeric envelope
Drug released through polymer

Figure 11: Swelling dosage form prior to administration

- **Unfolding GRDFs:**

Unfolding devices are characterized by different erodibility, mechanical properties, sizes and geometries These devices have the following properties: sufficient resistance to forces applied by the stomach, thus preventing rapid passage through the pylorus; allowance of free passage of food while in residence in the stomach; and desired in vivo circumference larger than 5 cm, to ensure gastroretentivity. The median GRT achieved is apparently prolonged and accordingly, may improve drug therapy. (Eytan K 2003)

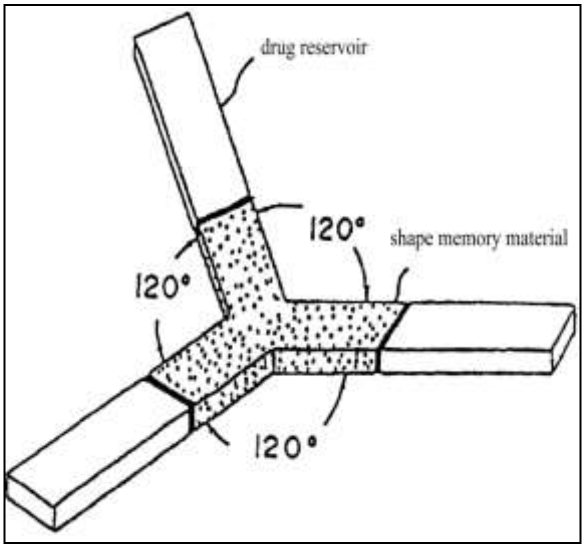

Figure 12: Unfolding dosage form

C. BIO/MUCOADHESIVE SYSTEMS:

Bio/Mucoadhesive systems bind to the gastric epithelial cell surface, or mucin, and extend the GRT by increasing the intimacy and duration of contact between the dosage form and the biological membrane. The concept is based on the self-protecting mechanism of the GIT. The epithelial adhesive properties of mucin are well known and have been applied to the development of GRDDS through the use of bio/Mucoadhesive polymers. The adherence of the delivery system to the gastric wall increases residence time at a particular site, thereby improving bioavailability. A bio/Mucoadhesive substance is a natural or synthetic polymer capable of adhering to a biological membrane (bioadhesive polymer) or the mucus lining of the GIT .They must be nontoxic and non-absorbable, form non-covalent bonds with the mucin–epithelial surfaces, have quick adherence to moist surfaces, easily incorporate the drug, offer no hindrance to drug release, have a specific site of attachment, and be economical. The binding of polymers to the mucin– epithelial surface can be subdivided into three broad categories:

Hydration-mediated adhesion:

Certain hydrophilic polymers tend to imbibe large amount of water and become sticky, thereby acquiring bioadhesive properties. The prolonged gastro retention of the bio/Mucoadhesive drug delivery system is further by the dissolution rate of the polymer.

Bonding-mediated adhesion:

The adhesion of polymers to a mucus or epithelial cell surface involves various bonding mechanisms, including physical–mechanical bonding and chemical bonding. Physical–mechanical bonds can result from the insertion of the adhesive material into the crevices or folds of the mucosa. Chemical bonds may be either covalent (primary) or ionic (secondary) in nature. Secondary chemical bonds consist of dispersive interactions (i.e., van der Waals interactions) and stronger specific interactions such as hydrogen bonds. The hydrophilic functional groups responsible for forming hydrogen bonds are the hydroxyl and carboxylic groups.

Receptor-mediated adhesion:

Certain polymers can bind to specific receptor sites on the surface of cells, thereby enhancing the gastric retention of dosage forms. Certain plant lectins such as tomato lectins interact specifically with the sugar groups present in mucus or on the glycocalyx. the median GRT achieved is apparently prolonged and accordingly, may improve drug therapy. The most promising excipients that have been used commonly in these systems include polycarbophil, Carbopol, lectins, chitosan etc. (Arvind 2003)

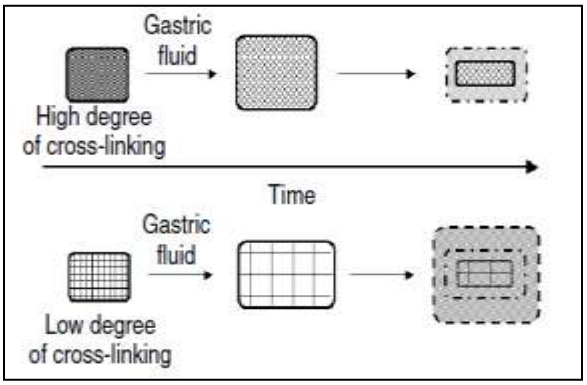

Figure 13: Relationship between the degree of cross-linking of the polymeric chains and the swelling behavior of swelling systems.

Evaluation:

Bioadhesion Studies and Duration of Buoyancy:

A tablet is attached to a stainless steel probe fixed to the mobile arm of the texture analyzer. The area of contact of gastric porcine mucosa is moistened with

50 µl of SGF. The mobile arm was lowered at a rate of 0.5 mm/s until a contact with the membrane was made. A contact force of 10 g was maintained for 300 s, after which the probe was withdrawn from the membrane. The peak detachment force was recorded as a measure of bioadhesion.

Determination of Buoyancy Duration:

The duration for which the formulation floats in the dissolution medium, in the upper one-third of the dissolution vessel, is determined periodically after every 15 min, by careful visual observation during the dissolution run.

Determination of Specific Gravity:

The specific gravity of tablets was determined by displacement method, using benzene as a displacing medium. A plethysmometer is employed to measure tablet density. Five tablets of known mass were dropped in wider mouth of the plethysmometer. The system is kept undisturbed for 1 min to let benzene displace the air in the pores of the tablets. The displacement in the volume of benzene in the side capillary is noted. Knowing the weight and volume occupied by the tablets, density of five tablets is determined. (AFSAR S 2011)

D. SUPERPOROUS HYDROGEL:

Superporous hydrogel (SPH) were originally developed as a novel drug delivery system to retain Drugs in the gastric medium. Superporous hydrogel composites have a combination of a high swelling rate and a ratio of more than 100 times the original weight of the dried matrix with substantial mechanical strength. These systems should instantly swell in the stomach and maintain their integrity in the harsh stomach environment, while releasing the pharmaceutical active ingredient. Conventional hydrogel have relatively small pore sizes, and reach by diffusion of aqueous media equilibrium-swelling after a period of several e.g. 12 h. In contrast, inside the SPHs water flows through an open channel system with pores of a few hundred micrometers, by capillary effect. The rapid swelling occurring within 20 min prevents premature emptying from the stomach by 'housekeeper wave'. (Hossein O 2007)

Hydroxy propyl methylcellulose (HPMC) is the most widely used hydrogel for this application. It gradually swells in the aqueous medium and controls drug release by both diffusion and erosion. These types of hydrogel are non-cross linked and ultimately dissolve over time in the presence of sufficient water or the swelling medium. Cross linked hydrogel have the ability to expand in aqueous environments up to 200 700 times their own weight in the dry state. The SPH in general are mostly characterized by their swelling and mechanical properties in different media. The swelling medium can be simulated gastric fluid. The swelling properties are measured by weight, volume and dimension at different time intervals.

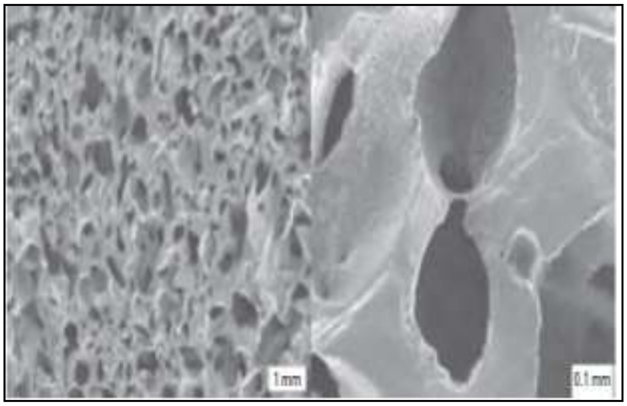

Figure 14: SEM cross-sectional picture of a typical Superporous hydrogel

E. HIGH-DENSITY SYSTEMS:

These systems, which have a density of ~3 g/cm^3, are retained in the rugae of the stomach and are capable of withstanding its peristaltic movements Sedimentation has been employed as a retention mechanism for pellets that are small enough to be retained in the rugae or folds of the stomach body near the pyloric region, which is the part of the organ with the lowest position in an upright posture. Dense pellets (approximately 3g/cm-^{3}) trapped in rugae also tend to withstand the peristaltic movements of the stomach wall. With pellets, the GI transit time can be extended from an average of 5.8–25 hours, depending on density than on the diameter of the pellets. Commonly used excipients are barium sulphate, zinc oxide, titanium dioxide and iron powder, etc. These materials increase density by up to 1.5–2.4g/cm-^{3}. (Garg R 2008)

Evaluation:

The various parameters that need to be evaluated for their effects on GRT of buoyant formulations can mainly be categorized into following different classes.

1. Galenic parameters: diametral size ('cut-off size'), flexibility and density of matrices.
2. Control parameters: floating time, dissolution, Specific gravity, content uniformity, and hardness and friability (if tablets).
3. Geometric parameters: shape.
4. Physiological parameters: age, sex, posture, food, bioadhesion.

Limitations of GRDDS:

1. Floating system is not feasible for those drugs that have solubility or stability problem in G.I. tract.
2. These systems require a high level of fluid in the stomach for drug delivery to float and work efficiently-coat, water.
3. The drugs that are significantly absorbed through out gastrointestinal tract, which undergo significant first pass metabolism, are only desirable candidate.
4. Some drugs present in the floating system causes irritation to gastric mucosa. (Archana M 2008)
5. In the case of bioadhesive systems, which form electrostatic and hydrogen bonds with the mucus, the acidic environment and the thick mucus prevent bond formation at the mucus–polymer interface.
6. Any dosage form designed to stay in the stomach during the fasted state must be capable of resisting the housekeeper waves of Phase III of the MMC.

Future Potentials:

Site specific drug delivery:

A floating dosage form is a feasible approach especially for drugs like riboflavin and furosemide which have limited absorption sites in upper small intestine. The absorption of furosemide has been found to be site-specific, the stomach being the major site of absorption, followed by the duodenum. Monolithic floating dosage form for furosemide, could prolong the GRT, and increase its bioavailability. Bilayer floating capsule has been used to achieve local delivery of misoprostol at the gastric mucosa level. It is a synthetic prostaglandin E_1analog approved and marketed in the US (as Cytotec®) for prevention of gastric ulcers caused by non-steroidal anti-inflammatory drugs (NSAIDs). Basically it replenishes the GI-protective prostaglandins that are depleted by NSAIDs. Thus, the controlled, slow delivery of misoprostol to the stomach provides sufficient local therapeutic levels and limits the systemic and intestinal exposure to the drug. This reduces the side effects. The prolonged gastric availability from a site directed delivery system may also reduce the dosing frequency.

Sustained Drug Delivery:

Drug absorption from oral CR dosage forms is often limited by the short GRT available for absorption. However, HBS type dosage forms can remain in the stomach for several hours and, therefore, significantly prolong the GRT of numerous drugs. These systems have a bulk density of less than 1 as a result of which they can float on the gastric contents. These special dosage forms are light, relatively large in size and do not easily pass through the pylorus.

A prolonged GRT is not responsible for the slow absorption of a lipophilic drug such as isradipine that has been achieved with a 'floating' modified-release capsule. This is because the major portion of drug release modified- release capsule took place in the colon, rather than in the stomach. However, the assumed prolongation in the GRT is postulated to cause sustained drug-release behavior. The administration of diltiazem floating tablets twice a day may be more effective compared to normal tablets in controlling the blood pressure of hypertensive patients. The duration of hypotensive effects was longer with floating tablets than that with normal ones. Madopar® HBS, has been shown to release levodopa for up to 8 h invitro, whereas the release from the standard Madopar® formulation is essentially complete in less than 30 min. Madopar® HBS behaves as a controlled / slow-release formulation of L-dopa and benserazide.

Sustained release floating capsules of nicardipine hydrochloride were developed and were evaluated.

Plasma concentration time curves showed a longer duration for administration (16 hours) in the sustained release floating capsules as compared with conventional MICARD capsules (8 hours). (Brahma N 2000)

Absorption Enhancement:
Drugs that have poor bioavailability because of site specific absorption from the upper part of the gastrointestinal tract are potential candidates to be formulated as floating drug delivery systems, thereby maximizing their absorption. (Archana M 2008)

Recent Advances:

Multi-unit floating alginate (Alg) microspheres using calcium carbonate were developed by Ninan Ma *et al.* To enhance the drug encapsulation efficiency and delay the drug release chitosan (Cs) was added. The gastrointestinal transit of the optimized floating sustained release microspheres was compared with that of the non-floating system manufactured from identical material using the technique of gamma-scintigraphy. A prolonged gastric retention time of over 5 h was achieved in the volunteers for the optimized coating floating microspheres. (Amit K 2011)

GRDDS, comprised mainly of floating, bioadhesive, and swellable systems, have emerged as an efficient means of enhancing the bioavailability and controlled delivery of drugs that exhibit an absorption window. The recent developments include the physiological and formulation variables affecting gastric retention, approaches to design single-unit and multiple-unit floating systems by prolonging the gastric emptying Marketed Products of GRDDS time of the dosage form. These systems not only provide controlled release of the drug for a prolonged period, but also present the drug in an absorbable form at regions of optimal absorption. These systems achieve this by retaining the dosage form in the gastric region, from where the drug is presented at the absorption window. This ensures maximal absorption of the drug for the desired period. The effect of GI physiology on drug delivery and the increasing sophistication of delivery technology will ensure the development of an increasing number of GRDDS to optimize delivery of drug molecules that exhibit regional variability in intestinal absorption. A novel floating controlled-release drug delivery system increases the gastric retention time of the dosage form and controls drug release. Floating matrix tablets are designed to prolong the gastric residence time after oral administration, at a particular site and controlling the release of drug especially useful for achieving controlled plasma level as well as improving bioavailability.

Brand name	Drug	Company & Country	Remarks
Modapar®	Levodopa (100 mg), Benserazide (25 mg)	Roche Products, USA	Floating CR Capsule
Valrelease®	Diazepam (15 mg)	Hoffmann- LaRoche, USA	Floating capsule
Liquid Gavison®	Al hydroxide (95 mg), Mg carbonate (358 mg)	Glaxo Smith Kline, India	Effervescent floating liquid alginate preparation
Topalkan®	Al-Mg antacid	Pierre Fabre Drug, France	Floating liquid Alginate preparation
Conviron®	Ferrous sulphate	Ranbaxy, India	Colloidal gel forming FDDS
Cifran OD®	Ciprofloxacin (1 gm)	Ranbaxy, India	Gas-generating floating tablet
Oflin OD®	Ofloxacin (400mg)	Ranbaxy, India	Gas generating floating tablet
Cytotec®	Misoprostal (100 mcg/200 mcg)	Pharmacia, USA	Bilayer floating capsule

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COLON SPECIFIC DRUG DELIVERY SYSTEM

Bhuruk Manisha, Pawar Yogesh

Introduction:

During the last two decade there has been interest in developing site-specific formulations for targeting drug delivery to the colon. It is a very challenging task for pharmaceutical technologist to deliver the drugs in the colon, in conditions where a drug needs to be protected from the hostile environment of upper GIT. The colon is a site where both local and systemic drug delivery can take place. A local means of drug delivery could allow topical treatment of inflammatory bowel disease, e.g. ulcerative colitis, crohn's disease, colon polyps. Such inflammatory conditions are usually treated with glucocorticoids and sulphasalazine. Treatment might be more effective if the drug substances were targeted directly on the site of action in the colon. Lower doses might be adequate and, if so, systemic side effects might be reduced. A number of other serious diseases of the colon, e.g. colorectal cancer, might also be capable of being treated more effectively if drugs were targeted on the colon. Site-specific means of drug delivery could also allow oral administration of peptide and protein drugs, (insulin, growth hormones) which normally become inactivated in the upper parts of the gastrointestinal tract due to presence of peptidase enzyme. The colon is rich in lymphoid tissue, uptake of antigens into the mast cells of the colonic mucosa produces rapid local production of antibodies and this helps in efficient vaccine delivery. Additionally, the colon has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs. Colon-specific systems could also be used in conditions in which a diurnal rhythm is evident, e.g. asthma, rheumatic disease, ulcer disease and ischemic heart disease. The incidence of asthmatic attacks is, for example, greatest during the early hours of the morning. Because dosage forms remain longer in the large intestine than in the small intestine, colon-specific formulations could be used to prolong drug delivery.

Colon delivery can be accomplished by oral or rectal administration. Rectal dosage form such as suppositories and enemas are not always effective as high variability in the distribution of these forms is observed. Suppositories are effective in only the rectum because of the confined spread. Enema Solutions can show their result after topical treatment to only the sigmoid and descending Colon. Hence oral administration is preferred. For oral administration to succeed, however many physiological barriers have to overcome. Absorption or degradation of active ingredients in the upper part of gastrointestinal tract is main obstacle and must be circumvented for successful colonic drug delivery.

Slow movement of material through the colon allows a large microbial population to thrive there. The colon microbiology is complex. Over 400 species of aerobic and anaerobic bacteria reside there, but anaerobic flora predominates. The number of anaerobic bacteria is approximately 10^{11} to 10^{12} colony forming unit per milliliter, resulting in almost one third of dry weight of the faeces in humans.

Anatomy and Physiology of Colon:

(Hota.A 2000)

Irrespective of therapy desire for local (colonic) or systemic delivery of the drug, the development and aim of the drug delivery to the colon remain same; firstly the drug must not be absorbed from other region of the GIT. Secondly it should not suffer considerable degradation in small intestinal lumen, thirdly release of the drug in the colon should be at quantitatively controlled rate and released drug should be absorbed from lumen of large intestine without any appreciable degradation in the lumen. In order to meet these priories a thorough knowledge of anatomy and physiology of GIT is required.

The GI tract is divided in to, stomach, small intestine, large intestine. The large intestine extended from the ileocecal junction to the anus with the length of about 1.5m (5ft) and diameter is of 6.5cm (2.5in) in adults and is divided in to three main parts.

- Colon
- Rectum
- Anal canal

Colon its self is made of seventh parts:

- Caecum
- The ascending colon
- The hepatic flexure
- The transverse colon

- The splenic flexure
- The descending colon
- The sigmoid colon

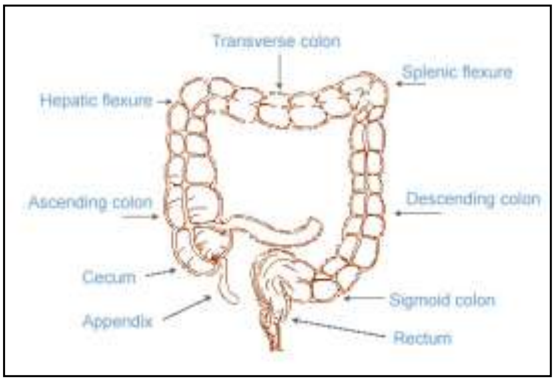

Figure 1: Schematic Representation of Human Colon

The opening from the ileum in to the colon is guarded by fold of mucus membrane is called as ileocecal sphincter (valve). This structure allows the material from the small intestine to pass in to the large intestine. Hanging below the ileocecal valve is Caecum a blind pouch about 6cm (2.5in) long. Attached to the caecum is twisted, coiled tube, measuring about 8cm (3 in) in length called vermiform appendix. Open end of the caecum merges with the ascending colon, which is retroperitoneal, ascend on right side of abdomen, reaches the under surface of liver, and turns abruptly to the left. Here it forms the right hepatic flexure. The colon continues across the abdomen to the left side as transverse colon, it is not retroperitoneal and curves beneath the lower end of the spleen on the left side as left splenic flexure and passes downward to the level of iliac crest as the descending colon also retroperitoneal structure. The sigmoid colon begins near the left iliac crest, project inward to the midline and terminates as the rectum at about the level of third sacral vertebra.

The colon serves four functions:

- Creation of suitable environment for the growth of colonic microorganism.
- Storage reservoir for faecal contents.
- Expulsion of content of the colon at an appropriate time, and
- Absorption of potassium and water from the lumen, concentrating faecal content.

Pathology of Colon:

Inflammatory bowel disease:

Chronic, relapsing, inflammatory disorders of unknown origin may have extra intestinal inflammatory manifestations often coexists with diverticular disease; the presence of Crohn’s in diverticular disease is suggested by fissuring ulcers, ulcers outside areas of active diverticulitis and fistulas other than colovesicle or colovaginal.

Crohn’s disease:

Transmural granulomatous disease affecting esophagus to anus but discontinuous, usually involves small intestine and colon Also known as regional enteritis, because it affects sharply delineated bowel segments called granulomatous colitis due to presence of granulomas primarily Western populations, prevalence 3 per 100,000, peaks in teens/twenties and ages 50-69 More common in whites, Jews, smokers; monozygotic twins have 30-50% concordance.

Colon Polyps:

Definition: mass protruding into lumen of gut.

Polyp with stalk may be due to traction on the mass. Polyps due to abnormal mucosal maturation, inflammation or architecture are non-neoplastic Polyps due to proliferation and dysplasia is neoplastic / adenomatous Polyploidy lesions may also be due to mucosal or submucosal tumors.

Inflammatory polyps:

Inflamed regenerating mucosa surrounded by ulcerated tissue; also granulation tissue overlying epithelium Associated with Crohn’s disease or ulcerative colitis; also amebiasis, schistosomiasis, ulcers, anastomotic sites Solitary small polyps in patients with inflammatory bowel disease may have bizarre stromal changes resembling sarcoma in a fibroblastic or granulation tissue stroma; no/few mitotic figures, no atypical mitotic figures, often zonation.

Familial adenomatous polyposis (FAP):

Also known as familial polyposis coli Defect in APC gene at 5q21, autosomal dominant trait with high degree of penetrance occurs in 1/8000 patients, 20% represent a new mutation100% progress to colonic adenocarcinoma, often in teens, most by thirties Patients have >100 colon polyps (usually thousands), beginning as teenagers, most are tubular adenomas May have cancer at time of diagnosis Polyps may also occur in stomach.

- **Carcinoma:**

In case of colonic cancers 98% are adenocarcinomas. cause of cancer deaths in US after lung cancer with 185,000 new cases and 55,000 deaths/year Affects 5% of US population during their lifetime most common GI tumor in US; less common in Africa, Asia, parts of South America Peak age 60-79; < 20% before age 50 .5% due to mutations in mismatch repair genes, 1% due to mutations in familial adenomatous polyposis gene. (Anil K.philip 2010)

Target Sites	Disease Conditions	Symptoms	Drugs and active agents
Topical / Local action	**Inflammatory Bowel Disease**		
	Crohn's disease	Diarrhea, Abdominal pain and cramping, blood in stool, ulcers, reduced appetite.	Hydrocortisone, Budenoside, Prednisolone, Sulfasalazine, Olsalazine, Mesalazine and Balsalazide, Infliximab
	Ulcerative colitis	Inflammation in the rectum, rectal bleeding, rectal pain	5-Amino salicylic acid, Sulfasalazine, Balsalazide, Infliximab, Azathioprine and mercaptopurine
	Irritable bowel syndrome	Abdominal pain or cramping, a bloated feeling, flatulence, diarrhea or constipation.	Dicyclomine, Hyoscine, Propantheline, Cimetropium, Mebeverine, Trimebutine, Scopolamine, Alosetron, Tegaserod
	Colorectal cancer	A change in bowel habits, narrow stools, rectal bleeding or blood in stool.	5-Flourouracil, Leucovorin, Oxaliplatin, Irinotecan, bevacizumab and cetuximab
	Antibiotic associated colitis	Overgrowth of *Clostridium difficile* and its subsequent toxin production	clindamycin, broad-spectrum penicillin's (e.g., ampicillin, amoxicillin), and cephalosporin's
	Hirschsprung's disease	Constipation	Metronidazole, Vancomycin, Loperamide, Botulinum toxin
Systemic action	To prevent first pass metabolism of orally ingested drugs	----	Steroids
	Oral delivery of peptides	----	Insulin, hormone
	Oral delivery of vaccines	----	Typhoid
	Ulcerative colitis	Ulcerative proctitis, pancolitis	Prednisolone metasulfobenzoate, tixocortol pivalate, fluticasone propionate.

Diagnosis of inflammatory bowel disease:

Blood tests: An increased number of white blood cells may indicate the presence of inflammation. New blood tests that measure certain antibodies may make it easier to differentiate Crohn's disease from ulcerative colitis. Feces sample is also taken and examined for blood, infectious agents, or both.

Endoscopic technique: Flexible sigmoidoscopy and colonoscopy are endoscopic techniques. They are important in the diagnosis of both ulcerative colitis and Crohn's disease and both techniques involve snaking a fiber optic tube called an endoscope through the rectum to view the lining of the colon. The tissue sample may also be collected for a biopsy.The other diagnostic techniques of this disease are constellation of positive endoscopic, ratiographic, and histological findings with negative stool cultures. The differential diagnosis of IBS includes infectious colitis, celiac sprue, intestinal lymphoma, radiation enteropathy, NSAIDs use, and ischemic colitis.

- **Colonic microflora:**

A large number of anaerobic and aerobic bacteria are present the entire length of the human GI tract. Over 400 distinct bacterial species have been found, 20- 30% of which are of the genus bacteroids[7]. The upper region of the GIT has a very small number of bacteria and predominantly consists of gram positive facultative bacteria. The rate of microbial growth is greatest in the proximal areas because of high concentration of energy source.The metabolic activity of microflora can be modified by various factors such as age, GI disease, and intake of drug and fermentation of dietary residues. (colonic delivery formulation,Recent patent on drug delivery and formulation 2007)

pH differences in the colon:

On entry in to the colon, the pH dropped to 6.4 ± 0.5. The pH in the mid colon was found to be 6.6 ± 1 and in the left colon, 7.0 ± 1 and the values are shown in Table 2.

Gastrointestinal transit:

Gastric emptying of dosage form is highly variable and depends primarily on whether the subject is fed or fasted and on the properties of the dosage form such as size and density. The transit times of dosage forms in tract are shown in Table 3.

Table 2: Average pH of the GI Tract

Location	pH
1. Stomach	1.5 – 2.0
Fasted condition	3.0 – 5.0
Fed condition	5.0 – 6.5
2. Small intestine	6.0 – 7.5
Jejunum	6.4
Ileum	6.7 – 7.3
3. Large intestine	
Right colon	
Mid colon and	
Left colon	

Table 3: Gastrointestinal Transit time of contents

Organ	Transit Time (hr)
Stomach	<1(fasting)
Small intestine	>3(fed)
Large intestine	3-4
	20-30

Diseases affecting colonic transit have important implications for drug delivery, diarrhea increases colonic transit and constipation decreases it. The digestive motility pattern takes place when food is present in the stomach. It is said by regular, frequent contractions (about 4-5/min.) which effect the mixing intestinal contents and moving them towards the colon in short segments and lasts as long as food remains present in the stomach. The most frequent movements seen in the colon are very slow segmenting movements that typically occur every 30 minutes.

- **Drug absorption in the colon:**

Drugs are absorbed passively by either paracellular or transcellular route. Transcellular absorption involves the passage of drugs through cells and this is the route most lipophilic drugs takes, where paracellular absorption involves the transport of drug through the tight junction between cells and is the route most hydrophilic drug takes.

The colon may not be the best site for drug absorption since the colonic mucosa lacks well defined villi as found in the small intestine. The slower rate if transit in colon lets the drug stay in contact mucosa for a longer period than in small intestine which compensates much lower surface area.

The colon contents become more viscous with progressive absorption of water as one travels further through the colon. This causes a reduced dissolution rate, slow diffusion of drug through the mucosa.

Theoretically, drug absorption can occur along the entire GI tract, while in actuality, most drugs are absorbed in the duodenum and proximal jejunum. Recent studies have shown that some drugs (e.g. Theophyline and Metoprolol) continue to be absorbed in the colon.

- **Oral preparations:**

Solid formulations intended for targeted drug release into the lower gastrointestinal (GI) tract are beneficial for the localized treatment of several diseases and conditions, mainly inflammatory bowel diseases, irritable bowel syndrome and colon cancer. Also, because of their natural potential to delay or avoid systemic absorption of drug from the small intestine, colonic formulations can be utilized for chronotherapy of diseases which are affected by circadian biorhythms (e.g., asthma, hypertension and arthritis), and to achieve clinically significant bioavailability of drugs that are poorly absorbed from the upper parts of the gastrointestinal tract because of their polar nature and/or vulnerability to chemical and enzymatic degradation in the small intestine (e.g., peptides and proteins). The recent patent literature pertaining to various modified release (MR) formulation methods that are claimed to provide colonic delivery for a wide range of therapeutic agents. These technologies either utilize a single or a combination of two or more physiological characteristics of the colon, which includes pH, microflora (enterobacteria), transit time, and luminal pressure. Accordingly, these technologies may be grouped under four distinct classes;

1. pH-controlled (or delayed-release) system
2. Time-controlled (or time-dependent) system
3. Microbially-controlled system
4. Pressure-controlled system.

Among these, formulations that release drugs in response to colonic pH, entero-bacteria, or both are most common and promising. (Basit.A.W 2004)

- **Topical preparations**

Topical Preparations (foams, suppositories or enemas) plays major role in ulcerative colities, either alone or in combination with oral steroids. They should generally not be used once a patient requires high-dose oral or intravenous steroid therapy.

- **Old systemic and topical steroids:**

Synthetic glucocorticoids such as prednisone, prednisolone, methyl-prednisolone, hydrocortisone, and ACTH are the most commonly used traditional corticosteroids in the treatment of ulcerative colitis.

Why colon is targeted drug delivery needed?

- Targeted drug delivery to the colon would ensure direct treatment at the disease site, lower dosing and fewer systemic side effects.
- Site-specific or targeted drug delivery system would allow oral administration of peptide and protein drugs, colon-specific formulation could also be used to prolong the drug delivery.
- Colon-specific drug delivery system is considered to be beneficial in the treatment of colon diseases.
- The colon is a site where both local or systemic drug delivery could be achieved, topical treatment of inflammatory bowel disease, e.g. ulcerative colitis or Crohn’s disease. Such inflammatory conditions are usually treated with glucocorticoids and sulphasalazine (targeted).
- A number of others serious diseases of the colon, e.g. colorectal cancer, might also be capable of being treated more effectively if drugs were targeted to the colon.

Formulations for colonic delivery are also suitable for delivery of drugs which are polar and/or susceptible to chemical and enzymatic degradation in the upper GI tract, highly affected by hepatic metabolism, in particular, therapeutic proteins and peptides. (M.k.chourasia 2003)

Factors Affecting Colonic Drug Delivery:

A. Physiological Factors
B. Pharmaceutical Factors

A. Physiological Factors:

a) pH of the colon:

The pH of the GI tract is subjected to both inter and intra subject variations, the table given below gives an overview of the pH of the GI tract, Radiotelemetry has been use to measure the gastrointestinal pH in healthy human subject.

Interspecies variability in pH is major concern when developing and testing colon specific drug delivery system in animal and applying the information to the humans. Colonic pH has been shown reduced in disease. The mean pH in the group of 7 patients with untreated ulcerative colitis 4.7 where as in 5 patients receiving the treatment it was 5.5. The in vitro fermentation of pharmaceutical polysaccharides such as isapghula and guar gum resulted in reduction of pH in presence of faecal bacteria

b) Gastrointestinal transit:

The drug delivery system first enters in to stomach and small intestine via mouth then reach colon. The nature and pH of gastric secretion and gastric mucosa influence the drug release and absorption in order to successfully reach colon in an intact form the drug delivery system should surpass the barrier in the stomach and small intestine. In fasted state the motility proceeds through four phase s occurring in stomach and small intestine that span over period of 2 – 3 hrs. Phase I is quiescent period of 40 – 60 min. Phase II consist of intermittent contraction for a period of 40 – 60 min. Phase III is period of intestine contraction sweeping material out of the stomach and down the small intestine followed by phase fourth with contraction dissipating. The feeding state affects the normal pattern by irregular contraction activity.

c) Small Intestinal Transit:

Normally the small intestinal transit is not influence by the physical state, size of the dosage form and the presence of food in the stomach. The mean transit time of dosage form is about 3 – 4 hrs to reach the ileocecal junction and the time period consistent. The dosage form exposed to enzyme such as esterase, lipase, amylase, protease, nuclease and brush border enzymes (glucosidases, disccharidases and peptidase) present in the small intestine. The release of the drug from the prodrug based system and the stability of the peptide can be affected by bacterial content in ileum.

d) Colonic Transit:

The bioavailability of drug, release from the dosage form can be highly influenced by colonic transit time shows considerable variability. Various factors like gender and size of the dosage form, and physiological condition such as stress, presence of food and the disease state influence the colonic transit time. Small particle and solution pass slowly through proximal colon and in human beings, men show shorter transit time than women. Small variation in dietary fibre and age does not alter the transit time, significantly. The colonic transit time of capsule in adult is 20 – 30 hrs, the transit rate being independent of capsule density and volume. Improve residence time with subsequent longer transit time and the contact of dosage with micro flora in the colon may govern release and absorption of the drug from the dosage form.

e) Gastric Emptying:

Generally, in fasted state, gastric emptying is fastest and most consistent. Emptying complete from 5 – 10 min. up to 2 hrs, depending on the phase of stomach at the time of drug administration. Gastric emptying can be considerably showed by fed state for drug delivery to the systemic vasculature, small transit time in the stomach to reduce random distribution of particulate drug through intestine is preferable. The residence time in stomach is important for single unit sustained release system like tablets, which are designed to deliver drug in large intestine. Such a system may release the drug at distance locus from the colon.

f) Colonic Microflora and Enzymes:

The human alimentary canal is highly populated with a bacteria & other Microflora at both ends that is the oral cavity & colon / rectum. Micro-organism of oral cavity, normally do not affect oral drug deliver system, however, gut Microflora of the colon offers a number of implications in health & treatment of disease such as IBD. The concentration of gut micro-flora rises considerably in the terminal ileum to reach extra ordinary high level (10^{-11} to 10^{-12} CFU/ml) in the colon. The colon micro-flora predominantly consists of *bacterides, Bifidobacteruim, Eubacterium, Peptococcus, Peptostreptococcus, Ruminococcus, Propionibacterium, Veillonella & Clostridium,* including important facultative *E.coli & lactobacill.*

The enzyme catalyzed metabolic reaction carried out by enzymes & secretary products released from the micro-flora can be used to deliver drug to colon. The oxidation reduction potential in colon is about 200mV. Azoreductase produced by the colonic micro-flora plays an important role in the development of number of delivery system, particularly in catalyzing the release of 5 –amino salicylic acid (5ASA) from a variety of prodrug. The reduction of azo bond is O_2 sensitive reaction apparently mediated by low molecular weight carrier with E_0 = -200 to -350mV.Other enzyme which trigger release of drug in the colon are glycosidase & glucoronidases produced by *lactobacilli, bacterides& Bifidobacterium.* The activity of enzyme is associated with the concentration of bacteria & particular region. The composition & population of the intestinal micro-flora remains constant under normal conditions but varies in disease state. Colon drug delivery is significantly affected by the alteration of colonic micro-flora due to oral administration of antibiotics during infectious disease. The metabolic activity of micro-flora is also dependant on fermentation of dietary residue. In some cases, the metabolic product of micro-flora can inactivate drug or potentiate their side effects.

g) Colonic Absorption:

The surface area of the colon is much less compared to the small intestine, and hence not ideally suited for absorption. Despite this limitation, the colon is considered for a drug delivery because the environment is devoid of endogenous digestive enzyme other than from microbial origin & the residence time of colon can be as long as 10 to 24 hours. Little mixing in the colon makes it possible to create local environment with optimal absorption condition. The higher viscosity of colonic content delays the diffusion of drug from the lumen to mucosa. The absorption is influenced by the transport of water, electrolyte and ammonia across the mucosa, & it is more in proximal colon than distal colon. The absorption properties of colon are generally studied by in vitro monolayer of the colon carcinoma cell lines, everted sacks & colonoscopy. The mucus layer at epithelial surface can be formidable physical barrier results in either drug mucus binding or drug mucus repulsion. The lipid bilayer of individual epithelium colonocytes & occluding junction complex between the colonocytes provide a physical barrier to impede colonic drug absorption.

Drug molecules pass from the apical to basolateral surface of the epithelial cells by:

1) Passing through colonocytes (transcellular transport), or
2) Passing between adjacent colonocytes (paracellular transport)

Small amphipathic drug may pass this barrier through transcellular transport. However, transit through cell cytoplasm may result in its extensive enzymatic extraction & its degradation. Paracellular transport may be the most promising means of general drug absorption in the colon. Since the membrane fluidity of proximal colonocytes is higher than distal colonocytes; drug can easily pass via passive absorption process in the proximal colon. Additionally, carrier mediated uptake of a drug in the colon is not extensive & usually relates to the metabolic events of resident bacteria. Receptor mediated endocytosis & pinocytosis could, however lead to transcellular transport of drug.

The colonic epithelial permeability of many drugs can be modified by the use of absorption enhancers. Absorption enhancers facilitate effective absorption through various mechanisms:

1) Disruption of intracellular occluding junction complex opens the paracellular route.
2) Modification of epithelial permeability by denaturing membrane proteins.
3) Modification of lipid protein interaction & disruption of the integrity of lipid barrier by colonic enterocytes.

Several absorption enhancers are conventionally used to enhance the absorption of polar drugs. Similarly, protease inhibitor such as aprotinin & bacitracin enhance the absorption of peptide & proteins, by preventing their destruction from aminopeptidase’s activity. The use of absorption enhancer in pharmaceutical formulation is limited since they are nonspecific in action produced local irritation & lead to irreversible changes in permeability of colon.

h) Gastrointestinal Disease State:

General intestinal disease such as IBD, crohn’s disease, constipation, diarrhea & gastroenteritis may affect the release & absorption properties of colon specific drug delivery system. Most of the disease associated with nausea & vomiting may expel the drug content. In antibiotic related colitis condition, plaque formation on the mucosa may interfere with absorption of drug. The antibiotic induced depression intestinal motility, particularly the muscularis mucosa, may enhance antibiotic resistant bacterial growth leading to colitis. It may initiate or exacerbate diarrhea by altering the epithelial function of distal colon. In case of severe crohn’s disease, bacterial enzymes utilize for colon specific drug delivery are decreased or become inactive. Azoreductase & nitroreductase activity of bacteria is higher during recovery from pouchitis. While designing colon specific drug delivery system, these factors should be taken into consideration.

B. Pharmaceutical Factors:

a) Drug candidates:

Drug which shows poor absorption from the stomach or intestine including peptide drugs (amylin, calcitonin, cyclosporine, vasoprossin, insulin, interferons etc.), are most suitable for colon specific drug delivery system. The drug used in the treatment of IBD, ulcerative colitis, diarrhea (metronidazole, tinidazole), colon cancer (5-flurouracil, methotrexate, avastin) and the drug use treatment of colon polyps like celecoxib are ideal candidates for local colon delivery. Sulphasalazine & 5ASA are widely used drugs for the treatment of IBD & other drugs include dexamethasone, prednisolone, hydrocortisone, budenoside.

b) Drug Carriers:

The selection of carrier for particular drug candidate depends on physico-chemical nature of the drug as well as the disease for which the system is to be used. The factor such as chemical nature, stability & partition coefficient of the drug & the type of absorption enhancer has chosen influence the carrier selection. Moreover, the choice of drug carrier depends on the functional groups of the drug molecule. For example, aniline or nitro groups on a drug may be used to link it to another benzene group through an azo bond. The carrier which contains additives like polymer (may be used as matrices and hydrogel or coating agents) may influence the release properties and efficacy of the systems. (Kothawade.P.D n.d.)

• Targeting Approaches to the Colon:

A. Covalent Linkage of the Drug with a Carrier:

It involves the formation of a covalent linkage between drug and carrier in such a manner that upon oral administration the moiety remains intact in the stomach and small intestine. This approach chiefly involves the formation of prodrug, which is a pharmacologically inactive derivative of a parent drug molecule that requires spontaneous or enzymatic transformation in the biological environment to release the active drug. Formation of prodrug has improved delivery properties over the parent drug molecule. The problem of stability of certain drugs from the adverse environment of the upper GIT can be eliminated by prodrug formation, which is converted in to parent drug molecule once it reaches in to the colon. Site specific drug delivery through site specific prodrug activation may be accomplished by the utilization of some specific property at the target site, such as altered pH or high activity of certain enzymes relative to the non-target tissues for the prodrug-drug conversion**.**

1. Azo bond conjugates*:* The intestinal microflora is characterized by a complex and relatively stable community of microorganism, many with physiological functions, which play vital roles in health and disease. In addition to protection of the patient against colonization of the intestinal tract by potentially pathogenic bacteria, the indigenous microflora are responsible for a wide variety of metabolic processes, including the reduction of nitro and azo groups in environmental and therapeutic compounds.

Sulphasalazine was introduced for the treatment of rheumatoid arthritis and anti-inflammatory disease. Chemically it is salicylazosulphapyridine (SASP), where sulfapyridine is linked to a salicylate radical by an azo bond. When taken orally, only a small proportion of the ingested dose is absorbed from the small intestine and the bulk of the Sulphasalazine reaches the colon intact. There it is split at the azo bond by the colonic bacteria with the liberation of sulphapyridine (SP) and 5-ASA (Figure3). However sulphapyridine is seems to be responsible for most of the side effects of Sulphasalazine and hence various new approaches for the treatment of IBD have emerged.

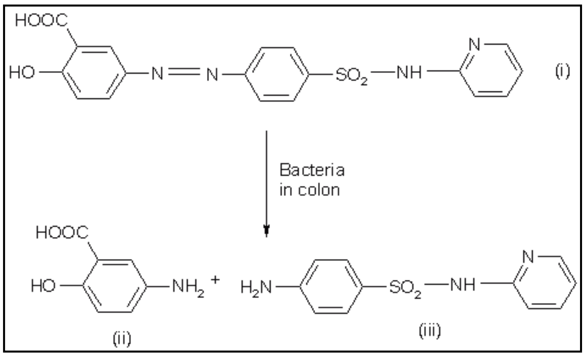

Figure 2: Hydrolysis of sulfasalazine (i) in to 5-aminosalicylic acid (ii) sulfapyridine (iii)

2. Glycoside conjugates:

Steroid glycosides and the unique glycosidase activity of the colonic microflora form the basis of a new colon targeted drug delivery system. Drug glycosides are hydrophilic and thus, poorly absorbed from the small intestine. Once such a glycoside reaches the colon it can be cleaved by bacterial glycosidases, releasing the free drug to be absorbed by the colonic mucosa.

The major glycosidases identified in human feces are β -D-galactosidase,-D-glucosidase, □-L-arabinofuranosidase, and β -D-xylopyranosidase[47]. These enzymes are located at the brush border and hence access to the substrate is relatively easy. In the plant kingdom numerous compounds are found as glycosides. Certain drugs act as glycon and can be conjugated to different sugar moieties which results in the formation of glycosides. Due to the bulky and hydrophilic nature of these glycosides, they do not penetrate the biological membrane upon ingestion[48]. Various naturally occurring glycosides, e.g. the sennosides, have been used for laxative action for ages. When taken orally, intact sennosides are more efficient as laxative than sugar free aglycones. These sennosides are activated by colonic microflora to generate rhein anthones, which gives the desired laxative effect[49]. Glycosidase activity of the GIT is derived from anaerobic microflora in the large bowel or the sloughed or exfoliated cells of the small intestine[50,51]. Friend and Chang prepared dexamethason-21- b -glucoside (Figure 4) and prednisolone-21- b -glucoside for delivery of these steroids to the colon. Hydrolysis of prodrugs by b -glucosidase and fecal homogenates *in vitro* released the free steroids. Glucosides were administered to rats intragastrically to determine when and where the free steroids were released. Unmodified dexamethasone and prednisolone were also given to rats intragastrically to compare absorption of the glucosides with the free steroids. Both glucosides were found to reach the rat lower intestine in 4-5 h, where they were rapidly hydrolyzed, releasing the free steroids.

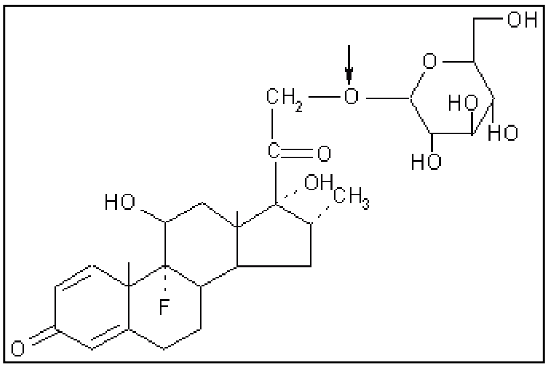

Figure 4: Dexamethasone-21-b-D-glucoside (Arrow shows site of action of glycoside)

In vivo studies on dexamethasone- β -D-glucoside revealed that nearly 60% of an oral dose of glucoside reached the caecum whereas in case of prednisolone- β -D-glucoside, only 15% reached to the caecum. When free steroids were administered orally, they were almost absorbed in the small intestine and less than 1% of oral dose reached at the colon.

3. Glucuronide conjugates:

Glucuronide and sulphate conjugation is the major mechanisms for the inactivation and preparation for clearance of a variety of drugs. Bacteria of the lower GIT, however, secrete β -glucuronidase and can deglucuronidase a variety of drugs in the intestine[53]. Since the deglucuronidation process results in the release of active drug and enables its reabsorption, glucuronide prodrugs would be expected to be superior for colon targeted drug delivery. Morphine-dependent rats were used to evaluate the effects of the narcotic antagonists, naloxone and nalmefene, and their glucuronide conjugates on the gastrointestinal tract and various parameters of brain-mediated withdrawal. When administered subcutaneously nalmefene hydrochloride caused a dose-dependent tail skin temperature increase, whereas nalmefene glucuronide was ineffective. Nalmefene precipitated brain-mediated morphine withdrawal at doses as low as 10 mg/kg, whereas nalmefene glucuronide was ineffective at doses as high as 1 mg/kg. After per oral administration of the drugs, naloxone hydrochloride and nalmefene hydrochloride caused diarrhea, withdrawal behavior and tail skin temperature responses by 15 minutes. In contrast, after per oral administration of the glucuronide conjugate of either narcotic antagonist, diarrhea was delayed for 75 to 203 minutes. This latency probably reflects the required transit time to the lower gastrointestinal tract. About 0.2 to 0.5% of the dose of the narcotic antagonist administered orally as the glucuronide was absorbed systemically. These results indicate that per oral administration of the glucuronide conjugates of naloxone and nalmefene results in delivery of the narcotic antagonists to the colon. Haeberlin et al prepared a dexamethasone- b-D-glucuronide, prodrug.

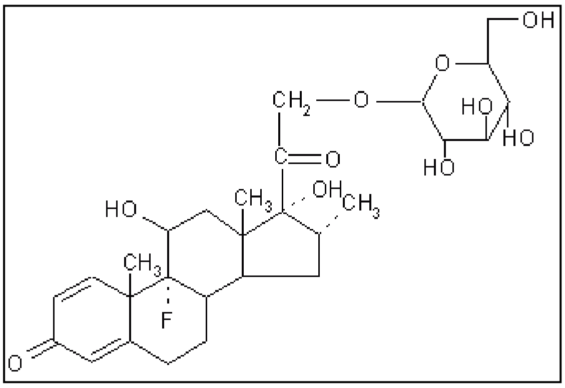

Figure 3: Dexamethasone- b -D-glucuronide.

4. Cyclodextrin conjugates:

Cyclodextrin are cyclic oligosaccharides consisted of six to eight glucose units through a 1 - 4 glucosidic bonds and have been utilized to improve certain properties of drugs such as solubility, stability and bioavailability. The interior of these molecules is relatively lipophilic and the exterior relatively hydrophilic, they tend to form inclusion complexes with various drug molecules.They are known to be barely capable of being hydrolyzed and only slightly absorbed in passage through the stomach and small intestine; however, they are fermented by colonic microflora in to small saccharides and thus absorbed in the large intestine. Cyclodextrin is capable of alleviating the undesirable properties of drug molecules in various routes of administration through the formation of inclusion complexes. In an oral drug delivery system, the hydrophilic and ionizable cyclodextrin can serve as potent drug carriers in the immediate release and delayed release-formulations, respectively, while hydrophobic cyclodextrin can retard the release rate of water-soluble drugs. Since cyclodextrin are able to extend the function of pharmaceutical additives, the combination of molecular encapsulation with other carrier materials will become effective and a valuable tool in the improvement of drug formulation. Moreover, the most desirable attribute for the drug carrier is its ability to deliver a drug to a targeted site; conjugates of a drug with cyclodextrin can be a versatile means of constructing a new class of colon targeting prodrugs.

It has been proved through a study in healthy human volunteers cyclodextrin are meagerly digested in small intestine but are completely degraded by the microflora of the colon. Most bacterial strains that are isolated from human being are capable of degrading cyclodextrin. It has been proved by their ability to grow on cyclodextrin by utilizing them as the sole carbon source and by the stimulation of cyclodextrinase activity by as low as 2-4 h of exposure to cyclodextrin. This property of the drug may be exploited for the formation of colon targeted drug delivery systems. Several cyclodextrins conjugates have been prepared and the enantioselective hydrolysis has described.

5. Dextran conjugates:

Dextran ester prodrug was prepared and *in vitro* release revealed that release of naproxan from prodrug was several folds higher in caecum homogenates than in control medium or homogenates of the small intestine of pig. The bioavailability of naproxan after oral administration of a dextran T-70-naproxan ester prodrug in pigs was assessed by Harboe et al. Compared to the administration of an oral solution of an equivalent dose of naproxan the average absorption fraction for the conjugate amounted to 91%. It was established that several features of the prodrug indicated that naproxan was released from the prodrug prior to systemic absorption and that drug activation involved the action of one or more enzyme systems located in the gastrointestinal tract. It was observed in rabbits, the plasma concentration-time curves for the conjugate were characterized by an initial lag time of about 2-3 h, whereas naproxan was detected in plasma immediately after per oral administration of the drug compound per se. The distribution of the prodrug along the GIT at various times after conjugate administration was assessed qualitatively by HPLC analysis of conjugated and free naproxan in various segments of the GIT. From these experiments it was suggested that drug regeneration was effective in the bowel below the ileum.

6. Amino-acid conjugates:

Due to the hydrophilic nature of polar groups like -NH2 and -COOH, that is present in the proteins and their basic units (i.e. the amino acids), they reduce the membrane permeability of amino acids and proteins. Various prodrugs have been prepared by the conjugation of drug molecules to these polar amino acids. Non-essential amino acids such as tyrosine, glycine, methionine and glutamic acid were conjugated to SA. The salicyluric acid (the glycine conjugate of SA) was found to be metabolized to SA by the microorganisms of the intestinal flora of rabbit and dog. The prodrug was absorbed into the systemic circulation from the upper GIT and hence it was proved unsuitable for delivery of drugs to the colon.

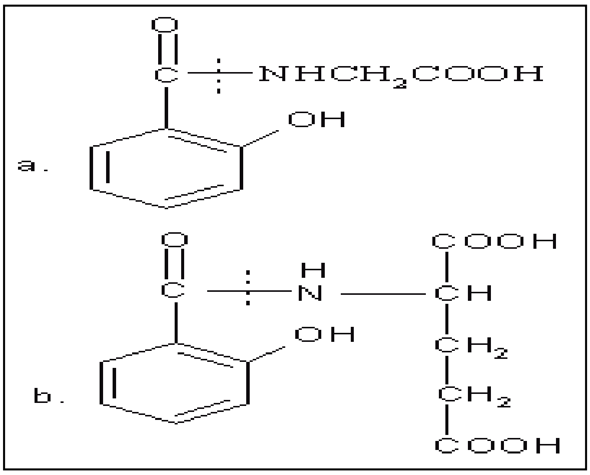

Figure 4: Glycine and glutamic acid conjugates of salicylic acid. (a) Salicyluric acid. (b) Salicyl-glutamic acid conjugate.

By increasing the hydrophilicity and chain length of the carrier amino acid and decreasing the membrane permeability of conjugate Nakamura et al. prepared salicylic glutamic acid conjugates (Figure 6). This conjugate showed splendid results with minimal absorption and degradation in the upper GIT and proved suitable for colon targeted delivery of SA.

- ## Approaches to Deliver the Intact Molecule to the Colon:

Coating with polymers:

The intact molecule can be delivered to the colon without absorbing at the upper part of the intestine by coating of the drug molecule with the suitable polymers, which degrade only in the colon.

Coating with pH-sensitive polymers:

The pH-dependent systems exploit the generally accepted view that pH of the human GIT increases progressively from the stomach (pH 1-2 which increases to 4 during digestion), small intestine (pH 6-7) at the site of digestion and it increases to 7-8 in the distal ileum. The coating of pH-sensitive polymers to the tablets, capsules or pellets provide delayed release and protect the active drug from gastric fluid. The polymers used for colon targeting, however, should be able to withstand the lower pH values of the stomach and of the proximal part of the small intestine and also be able to disintegrate at the neutral of slightly alkaline pH of the terminal ileum and preferably at the ileocecal junction. These processes distribute the drug throughout the large intestine and improve the potential of colon targeted delivery systems. While this release pattern can be studied *in vitro*, there is no real substitute for confirming reliable performance *in vivo* in man. The technique of gamma scintigraphy has become the most popular method to investigate the gastrointestinal performance of pharmaceutical dosage forms. The threshold pH commonly employed pH-sensitive polymers are Eudragit@ L100 (6.0 pH), Eudragit@ S 100 (7.0 pH), Eudragit@ L 30D (5.6 pH), Eudragit@ FS 30D (6.8 pH), Eudragit@ L100-SS (5.5 pH), polyvinyl acetate phthalate(5.0 pH), Hydroxypropyl methyl cellulose pthalate(4.5-4.8 pH), Hydroxypropyl methyl cellulose phthalate 50 (5.2 P^H), Hydroxypropyl methyl cellulose cellulose acetate cellulose acetate trimelliet (4.8 pH), cellulose acetate phthalate (5.0 pH).

The majority of enteric and colon targeted delivery systems are based on the coating of tablets or pellets, which are filled into conventional hard gelatin capsules. However, during the early stage of drug development some new chemical entities (NCE's) present a challenge in testing for efficacy due to instability in gastric fluids because of irritation in the GIT. The limited amount of drug substance available during the early stage often precludes the development of a coated pellet or tablet formulation. Since the coating process is independent of the capsule contents, there are clear advantages resulting from the ability to coat a capsule. Thus, the oral pharmacological and or therapeutic efficacy of the NCE can be determined without resorting to extensive, time consuming and in many instances, impossible at this point in the development of the NCE.

Coating with biodegradable polymers

The bioenvironmental inside the human GIT is characterized by the presence of complex microflora especially the colon that is rich in microorganisms that are involved in the process of reduction of dietary component or other materials. Drugs that are coated with the polymers, which are showing degradability due to the influence of colonic microorganisms, can be exploited in designing drugs for colon targeting. These bacterial degradable polymers especially azo polymers have been explored in order to release an orally administered drug in the colon. Actually, upon passage of the dosage form through the GIT, it remains intact in the stomach and small intestine where very little microbially degradable activity is present that is quiet insufficient for cleavage of polymer coating. Release of the drugs from azo polymer coated formulation is supposed to take place after reduction and thus degradation of the azo bonds by the azo reductase enzymes released by the azo bacters present in the colonic microflora. Since the concept of this strategy is based on the metabolic activity of azo reductase produced by azo bacters of colon, the bacterial degradation of polymeric coating may be effected by several other factors e.g. dietary fermentation precursors, type of food consumed and co-administration of chemotherapeutic agents. Administration of antibiotics may result in the partial or complete destruction of colonic microflora, which adversely affect the release of bioactive agents.

Linear-type-segmented polyurethanes containing an azo group in the main chain have been synthesised as coating material. Since this polymer was degraded specifically by the action of intestinal flora, the dosage form coated with this polymer would be effective for colon targeting of orally administered drugs. However, this poly-urathane based on m-xylene diisocynate (XDI), was soluble only in limited solvents and has been thought to be clinically inapplicable due to the trace amount of remaining solvent. Therefore Yamaoka et al. Synthesised a segmented polyurethane containing azo aromatic groups in the main chain by ratio of isophorone diisocyanate with a mixture of m, m'-di (hydroxymethyl) azobenzene, poly (ethylene glycol), and 1, 2-propanediol. This polyurethane was soluble in various solvents and showed a good coating and film-forming property. A solution-cast film of this polyurethane was found to be degraded in a culture of intestinal flora with the azo group reduction to hydroazo groups, not to amino groups. The film degradation, therefore, was attributed to the decreased cohesive energy in the hydroazo polymer compared with that in the original azo polymer. Then, the drug pellets containing water-soluble drugs were undercoated with (carboxymethyl) (ethyl) -cellulose and over coated with the azo polymer in order to examine the drug-releasing profiles in the culture of intestinal flora.

Embedding in matrices:

The drug molecules are embedded in the polymer matrix. The polymers used for this technique should exhibit degradability in the colon for liberation of entrapped drug.

Embedding in biodegradable matrices and hydrogels:

Polysaccharides, the polymer of monosaccharide retains their integrity because they are resistant to the digestive action of gastrointestinal enzymes. The matrices of polysaccharides are assumed to remain intact in the physiological environment of stomach and small intestine but once they reach in the colon, they are acted upon by the bacterial polysaccharidases and results in the degradation of the matrices. This family of natural polymers has an appeal to the area of drug delivery as it is comprised of polymers with a large number of derivatizable groups, a wide range of molecular weights, varying chemical compositions, and for the most part, a low toxicity and biodegradability, yet a high stability. The most favorable property of these materials is that they are already approved as pharmaceutical excipients. A large number of polysaccharides such as amylose, guar gum, pectin, chitosan, inulin, cyclodextrins, chondroitin sulphate, dextrans and locust bean gum have been investigated for their use in colon targeted drug delivery systems. The most important fact in the development of polysaccharide derivatives for colon targeted drug delivery is the selection of a suitable biodegradable polysaccharide. As these polysaccharides are usually soluble in water, they must be made water insoluble by crosslinking or hydrophobic derivatisation. Very important is an optimal proportional of the hydrophobic and hydrophilic parts respectively and the number of free hydroxy groups in the polymeric molecule.

Various biodegradable polymers used in colon specific drug delivery as matrix former are as follows; Amylose, Arabinogalactan, Chitosan, Chondroitin Sulphate, Cyclodextrin, Dextran, Guar gum, Pectin, Xanthan gum, Xylen etc.

Embedding in pH-sensitive matrices:

Extrusion-spheronization and pelletization have been used for the preparation of pH-sensitive matrix pellets for colon targeted drug delivery. The authors studied the effects of three independent variables (amounts of Eudragit® S, citric acid and spheronizing time) on pellet size, shape (roundness and aspect ratio), and drug release was studied with central composite design. Nykanen et al. Used ibuprofen as model drug and Eudragit® S and Aqoat AS-HF as enteric polymers for developing site-specific systems for release of a drug in the lower part of the small intestine or in the colon. The target of this study was to investigate whether drug release rate from enteric matrix granules could be influenced by using organic acids as excipients. It was concluded that although inclusion of an organic acid in a formulation retarded *in vitro* release of the model drug, no corresponding effect was evident in case of *in vivo* studies.

Timed Release Systems:

This approach is based on the principle of delaying the release of the drug until it enters into the colon. Although gastric emptying tends to be highly variable, small intestinal transit time is relatively constant or little bit variation can be observed. The strategy in designing timed-released systems is to resist the acidic environment of the stomach and to undergo a lag time of predetermined span of time, after which release of drug take place. The lag time in this case is the time requires to transit from the mouth to colon. The first formulation introduced based on this principle was Pulsincap®[81]. It is similar in appearance to hard gelatin capsule; the main body is made water insoluble (exposing the body to formaldehyde vapour which may be produced by the addition of trioxymethylene tablets or potassium permanganate to formalin or any other method). The contents are contained within a body by a hydrogel plug, which is covered by a water-soluble cap. The whole unit is coated with an enteric polymer to avoid the problem of variable gastric emptying. When the capsule enters the small intestine the enteric coating dissolves and the hydrogels plug starts to swell, the amount of hydrogel is such adjusted that it pops out only after the stipulated period of time to the release the contents. The viability of such a system in human volunteers has been confirmed on the basis of evaluation studies. In another method, an organic acid (succinic acid) was filled into the body of a hard gelatin capsule as a pH-adjusting agent together with the drug substance. The joint of the capsule was sealed using an ethanolic solution of ethylcellulose. The capsule was first coated with an acid soluble polymer (Eudragit® E), then with a hydrophilic polymer HPMC and finally enterically coated with Eudragit® L. After ingestion of the capsule, the outermost enteric layer of the coating prevents drug release in the stomach. Enteric layer and hydrophilic layers dissolve quickly after gastric emptying and water starts entering the capsule. When the environmental pH inside the capsule decreases by the dissolution of organic acid, the acid soluble layer dissolves and the enclosed drug is quickly released. Therefore, the onset time of drug releases in the intestine can be controlled by the thickness of acid soluble layer.

Redox-Sensitive Polymers:

Analogues to azo bond cleavage by intestinal enzymes, novel polymers that hydrolyzed nonenzymatically by enzymatically generated flavins are being developed for colon targeting. Biodegradation of azo polymers has been extensively studied in the literature. It is suggested that both an intracellular enzymatic component and extracellular reduction exist. Under anaerobic conditions, bacterial azo reduction by enzymatically generated reduced flavins where the initial substrate thought to be involved in cellular electron transport requires the presence of NADPH as its electron source. As NADPH is oxidized, the electron mediator (reduced flavins) acts as an electron shuttle from the NADPH dependent flavoprotein to the azo compound. Molecular modeling of low molecular weight azo compounds revealed that reduction of the azo bond to the hydroazo intermediate requires a low electron density within the azo region, and thus substitution of electron-withdrawing groups will favor this reaction. Redox potential is an expression of the total metabolic and bacterial activity in the colon and it is believed to be insensitive to dietary changes. The mean redox potential in proximal small bowl is - 67 ± 90 mv, in the distal small bowl is -196 ± 97 mv and in the colon is -145 ± 72 mv.

Thus, microflora-induced changes in the redox potential can be used as a highly selective mechanism for targeting to the colon. Bragger et al. Carried out investigations into the azo reducing activity, which could enlighten some factors affecting the bacterial reduction (cleavage) of azo compounds. A common colonic bacterium, *Bacteroides fragilis* was used as test organism and the reduction of azo dyes amaranth, Orange II, tartrazine and a model azo compound, 4, 4'-dihydroxyazobenzene were studied. It was found that the azo compounds were reduced at different rates and the rate of reduction could be correlated with the redox potential of the azo compounds. 4,4'-Dihydroxyazobenzene (E1/2 -470 mV) was reduced at the fastest rate of 0.75 mol l $^{-1}$ h $^{-1,}$ amaranth (E1/2 -568 mV) at 0.30 mol l-1 h $^{-1,}$ Orange II (E1/2 -648 mV) at 0.2 mol l $^{-1}$ h $^{-1}$ and tartrazine (E1/2 -700 mV) at 0.08 mol l-1 h-1. Similar observations were made with another colonic bacterium *Eubacterium limosum*.

Disulphide compounds can also undergo degradation due to the influence of redox potential in the colon. Noncrosslinked redox-sensitive polymers containing an azo and/or a disulfide linkage in the backbone have been synthesised. Radiological studies in dogs have investigated the *in vitro* behaviour of new polyurethane systems containing azo bonds.

Bioadhesive Systems:

Oral administration of some drugs requires high local concentration in the large intestine for optimum therapeutic effects. Dissolution of dosage form and simultaneous absorption from upper GIT lead to low intracolonic drug concentration as well as absorption of drugs result in the generation of side effects. Bioadhesion is a process by which a dosage form remains in contact with particular organ for an augmented period of time. This longer residence time of drug would have high local concentration or improved absorption characteristics in case of poorly absorbable drugs. This strategy can be applied for the formulation of colonic drug delivery systems. Various polymers including polycarbophils, polyurethanes and polyethylene oxide-polypropyline oxide copolymers have been investigated as materials for bioadhesive systems. Bioadhesion has been proposed as a means of improving the performance and extending the mean residence time of colonic drug delivery systems. *In vitro* bioadhesion has been confirmed from many studies and few reports are available in the literature regarding the in vivo bioadhesion studies. Kakoulides et al. synthesized Azo-networks based on an acrylic backbone crosslinked with DVAB.

Coating with microparticles:

Many of the protozoans especially *Entamoeba histolytica* remains confined in the large intestine, which necessitates high intracolonic drug concentration. This goal is not fulfilling with the current available therapy, as they are based on the principle of releasing drugs into upper GIT that is systemically absorbed and generates side effects. Mirelman et al. Prepared and evaluated a formulation that was rather diverted from the mainstream of conventional therapy. It consisted of small silica particles (5-10 mm in diameter) covalently linked to a potent antiamoebic drug, 2-(4-aminophenoxymethyl)-5-nitro-1-methylimidazole. Silica-drug particles were injected into mice, hamsters and guinea pigs. It was found that trophozoites phagocytosed the particles *in vivo* and *in vitro*, followed by rapid cell death due to the released drug. Analysis of mouse serum revealed that no drug was absorbed from the intestine after placement of the drug-containing particles in the intestine. The antiamoebic activity of particles recovered from the intestine was almost fully retained. This novel antiamebic concept may be useful for luminal therapy for asymptomatic amebiasis and may minimize side effects and frequency of administration.

Osmotic Control drug delivery:

The OROS-CT (Alza Corporation) can be used to target the drug locally to the colon for the treatment of disease or to achieve systemic absorption that is otherwise unattainable. The OROS-CT system can be single osmotic unit or may incorporate as many as 5-6 push-pull units, each 4mm in diameter, encapsulated with in a hard gelatin capsule (Figure 7). Each bilayer push pull unit contains an osmotic push layer and a drug layer, both surrounded by a semipermeable membrane. An orifice is drilled through the membrane next to the drug layer. Immediately after the OROS-CT is swallowed, the gelatin capsule containing the push-pull units dissolves.

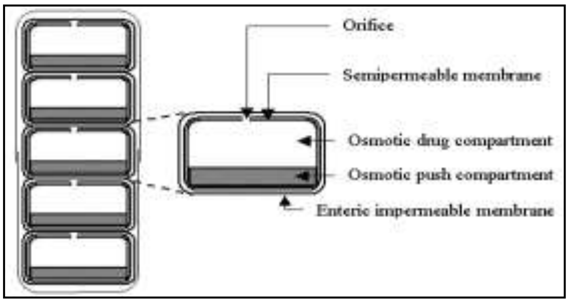

Figure 4: Cross section of the OROS-CT.

Because of its drug-impermeable enteric coating, each push-pull unit is prevented from absorbing water in the acidic aqueous environment of the stomach and hence no drug is delivered. As the unit enter the small intestine, the coating dissolve in this higher pH environment (pH >7), water enters the unit, causing the osmotic push compartment to swell and concomitantly creates a flowable gel in the drug compartment. Swelling of the osmotic push compartment forces drug gel out of the orifice at a rate precisely controlled by the rate of water transport through the semipermeable membrane. For treating ulcerative colitis, each push pull unit is designed with a 3-4 hour post gastric delay to prevent drug delivery in the small intestine. Drug release begins when the unit reaches the colon. OROS-CT units can maintain a constant release rate for up to 24 h in the colon or can deliver drug over an internal as short as 4 hour. (M.k.chourasia 2003)

Limitation and challenges in colon specific drug delivery system:

1. One challenge in the development of colon-specific drug delivery system is to establish an appropriate dissolution method in designing in-vitro system. Due to the rationale after a colon delivery system is quite diverse. As, a site for delivery offers a near neutral pH, reduced digestive enzymes activity, a long transit time, and increased responsiveness to absorption enhancers, hence targeting is complicated, with reliability and delivery efficiency.
2. Limiting factors for poorly soluble drug as the fluid contents in colon is much lower and it is more viscous than in upper part of GI tract, For successful delivery through thissite, drugs require to be in solution form before it arrives to colon and/or it should dissolve in luminal fluid of colon.
3. The resident microflora could also affect colonic performances via metabolic degradation of drug.
4. Lower surface area and relative ‘tightness’ of the tight junction in the colon can also restrict drug transport across the mucosa and into the systemic circulation.

- **Opportunities in colon targeted drug delivery:**

1. In the area of targeted delivery, the colonic region of the GI tract is the one that has been embraced by scientists and is being extensively investigated over the past two decades.
2. Targeted delivery to the colon is being explored not only for local colonic pathologies, thus avoiding systemic effects of drugs or inconvenient and painful trans-colonic administration of drugs, but also for systemic delivery of drugs like proteins and peptides, which are otherwise degraded and/or poorly absorbed in the stomach and small intestine but may be better absorbed from the more benign environment of the colon.
3. This is also a potential site for the treatment of diseases sensitive to circadian rhythms such as asthma, angina and arthritis. Moreover, there is an urgent need for delivery of drugs to the colon that reported to be absorbable in the colon, such as steroids, which would increase efficiency and enable reduction of the required effective dose.
4. The treatment of disorders of the large intestine, such as irritable bowel syndrome (IBS), colitis, Crohn’s disease and other colon diseases, where it is necessary to attain a high concentration of the active agent, may be efficiently achieved by colon-specific delivery.
5. The development of a dosage form that improves the oral absorption of peptide and protein drugs whose bioavailability is very low because of instability in the GI tract is one of the greatest challenges for oral peptide delivery.
6. The bioavailability of protein drugs delivered at the colon site needs to be addressed.
7. More research is focused on the specificity of drug uptake at the colon site is necessary. Such studies would significant

in advancing the cause of colon targeted drug delivery in future.

- **Advances in colon specific drug delivery system:**

Pulsatile drug delivery system:

A pulsatile drug release, where the drug is released rapidly after a well defined lag-time, could be advantageous for many drugs or therapies. Pulsatile release systems can be classified in multiple-pulse and single-pulse systems. A popular class of single-pulse systems is that of rupturable dosage forms. Other systems consist of a drug-containing core, covered by a swelling layer and an outer insoluble, but semi permeable polymer coating or membrane. The lag time prior to the rupture is mainly controlled by:

The permeation and mechanical properties of the polymer coating and the swelling behavior of the swelling layer. As is frequently found in the living body, many vital functions are regulated by pulsed or transient release of bioactive substances at a specific site and time. Thus it is important to develop new drug delivery systems to achieve pulsed delivery of a certain amount of drugs in order to mimic the function of the living systems, while minimizing undesired side effects. Special attention has been given to the thermally responsive poly (*N*isopropylacrylamide) and its derivative hydrogels. Thermal stimuli-regulated pulsed drug release is established through the design of drug delivery devices, hydrogels, and micelles.

Therefore, pulsatile drug delivery is one such systems that, by delivering drug at the right time, right place and in right amounts, holds good promises of benefit to the patients suffering from chronic problems like arthritis, asthma, hypertension.

- ➢ Methods for pulsatile drug delivery system:

(i) Capsular system:

Single unit systems are mostly developed in capsule form. The lag time is continued by a plug, which gets pushed away by swelling or erosion, and the drug is released as a pulse from the insoluble capsule body.

Example: Pulsincap® system. In this system a water insoluble body containing the drug formulation, system is closed with a swellable hydrogel. Plugged (insoluble but permeable and swellable) at open end. Upon contact with, gastrointestinal fluid or dissolution medium the plug swells pushing itself out of the capsule after lag-time.

Position and dimensions of plug and control lag-time. For rapid release of water insoluble drug effervescent or disintegrating agents are added. Plug material is generally made up of following:

a) Swellable materials coated with but permeable polymer (polymethacrylates).

b) Erodible compressed polymer (HPMC, polyvinyl alcohol).

c) Congealed melted polymer (glyceryl monooleate).

d) Enzymatically controlled erodible polymer (pectin).

(ii) Osmotic system: This system consists of a capsule coated with a semi permeable membrane. Inside the capsule was an insoluble plug consisting of osmotically active agent and the drug formulation. This system shows good *in-vivo* and *in vitro* correlation in humans and used to deliver methylphenidate to school age children for the treatment of Attention Deficit Hyper activity Disorder (ADHD), e.g.: Port® System. Another system is also based on expendable orifice that contain capsular system in which liquid drug is absorbed on highly porous particles. Drug releases through orifice of a semi permeable capsule supported by an expending osmotic layer after the barrier layer is dissolved. The Port® System (Port Systems, LLC) consists of a gelatin capsule coated with a semi permeable membrane (e.g., cellulose acetate) housing an insoluble plug (e.g., lipidic) and an osmotically active agent along with the drug formulation.

When in contact with the aqueous medium, water diffuses across the semi permeable membrane, resulting in increased inner pressure that ejects the plug after a lag time. The lag time is controlled by coating thickness.

(iii) Solubilisation (or) Erosion of membrane: These systems are based up on a drug reservoir surrounded with a soluble or erodible barrier layer that dissolves with time and the drug releases at once after the lag time. Eg. Time Clock® system.

The Time Clock system consists of solid dosage form coated with lipid barriers such as carnauba wax and beeswax along with surfactants like polyoxyethylene sorbitan monooleate. When this system comes in contact with the aqueous medium the coat emulsifies or erodes after the lag-time depending on the thickness of coat. The lag time of system is independent of the gastrointestinal motility, PH, enzyme and gastric residence.

(iv) Rupture of membrane: These systems are based up on a reservoir system coated with a rupturable membrane. The outer membrane ruptures due to the pressure developed by effervescent agents (or) swelling agent. Citric acid and sodium bicarbonate is incorporated as effervescent mixture in tablet core coated with ethyl cellulose, when system comes in contact with water it produces carbon dioxide gas which exerts pressure & after lag time rupture the membrane & rapid release of drug occurs. A reservoir system with a semi permeable coating is proposed especially with drugs with high first pass effect in order to obtain in-vivo drug pattern similar to the administration of several immediate release doses croscarmellose sodium starch glycollate or low substituted hydroxy propyl cellulose were used as swelling substances, which resulted in complete film rupture followed by rapid drug release. The lag time is controlled by composition of outer polymeric membrane. (al 2011) (Mor 2011)

- **Multiparticulate drug delivery systems:**

Multiparticulate drug delivery systems are reservoir type of oral dosage forms consisting of a collection of small discrete units, each exhibiting some desired characteristics. In these systems, the dosage of the drug substances is divided in a plurality of subunits, typically consists of thousands of spherical particles. Thus multiparticulate dosage forms are pharmaceutical formulations in which the active ingredient is present as a number of minute independent subunits. To deliver the recommended total dose, these subunits are filled into a capsule or compressed with additional excipients to form a tablet.

These systems show various advantages as well as disadvantages over single – unit systems, which are as follows:

Advantages:

1. Predictable, reproducible and short gastric residence time.
2. Less inter- and intra-subject variability.
3. Improve bioavailability.
4. Reduced adverse effects and improved tolerability.
5. Limited risk of local irritation.
6. No risk of dose dumping.
7. Flexibility in design.
8. Ease of combining pellets with different compositions or
9. Release patterns.
10. Improve stability.
11. Improve patient comfort and compliance.
12. Achieve a unique release pattern.
13. Extend patent protection, globalize product, and overcome
14. Competition.

- **Rationale behind designing the multiparticulate drug delivery systems:**

There are many reasons for designing and delivering drug as a multiparticulate system e.g.

1. To facilitate disintegration in the stomach. Shows better reproducible pharmacokinetic behavior then conventional (monolithic) formulations.

2. After disintegration, the individual subunit particles pass rapidly through the g.i.t. If these subunits have diameter of less than 2 mm, they are able to leave the stomach continuously, even if the pylorus is closed. These results in lower intra and inter individual variability in plasma levels and bioavailability.

3. Drug safety may also increased by using multiparticulate dosage forms.

Design of Multiparticulate Drug Delivery System:

The purpose of designing multiparticulate dosage forms is to develop a reliable formulation that has all the advantages of a single unit formulations and yet devoid of the danger of alteration in drug release profile and formulation behavior due to unit to unit variation (Rot et al., 2009).

Multiparticulate approaches tried for colonic delivery includes formulations in the form of pellets, granules, microparticles, nanoparticles, and beads. Because of their smaller particle size as compared to single unit dosage form these systems are capable of passing through the g.i.t. easily. Moreover, multiparticulate systems are to be more uniformly dispersed in the g.i.t. and also ensure more uniform drug absorption. As the units of multiparticulate systems are distributed freely throughout the g.i.t., their transport is affected to a lesser extent than single – unit formulations by the transit time of food .Multiparticulate systems are formulated as:

- **Reservoir system with rupturable polymeric coating:**

In these multiparticulate systems the reservoir devices are coated with a rupturable polymeric layer. Such systems comprised of many layers some layer contain drug substance, while others are rate – controlling polymers. The rupturing effect is achieved by coating the individual units with osmotic or swelling agents. Numerous release – profiles can be achieved using this approach – including sustained release of active pharmaceutical ingredients for absorption throughout the g.i.t. Time – delayed release of the drug as either a burst or sustained release profile can be achieved over a period of 1 – 12 h, with a lag – time of 4 – 10 h. The duration of drug release following the lag – time depends on the composition and thickness of the polymer barrier and the lag – time coating itself. The multiparticulate system provides an optimal release profiles for either single drugs or for a combination of drugs (Roy et al. 2009).

The first attempt to develop a time – dependent system for colon delivery was made by Ueda *et. al.* (1989, 1994). These inventers developed a time – controlled explosion system (TES) in which drug release is caused by explosion of a membrane after a definite time period (i.e. lag times), which is precisely programmed. TES were developed for both single and multiple – unit dosage forms.

In both cases, a core contains drug (model drugs used were metoclopramide hydrochloride, tiapride hydrochloride, sodium diclofenac and nilvadipine) plus an inert osmotic agent and suitable disintegrants. Individual units can be coated by a protective layer and then by a semipermeable layer, which is the rate controlling membrane for the influx of water into osmotic core. The osmotic pressure buildup by water ingress causes the core to explode, with an immediate release of the drug. The explosion of formulation can also be achieved through use of swelling agents. Hata et al., 1994 describes a similar time – controlled formulation. A four layered TES was developed where, drug was layered on an inner core (polystyrene balls or non – pareil sucrose beads), followed by a swellable layer (hydroxyl propyl cellulose) and an insoluble polymeric top layer (ethyl cellulose). An *In – vivo* study was carried out with this system for a new vasodilator drug (FK409) in conscious dogs. The drug appeared in the blood at 3 hand the maximum level was attained at 5 h was in accordance with *In – vitro* release profile. In bioavailability studies in human volunteers a 3h lag time to peak concentration at 5 h was obtained. These studies prove the system suitability for use in treatment of nocturnal symptoms of diseases. Blum (Blum et al., 2003; Kalantzi et al., 2009) described a controlled release oral dosage form of acetylsalicylic acid (aspirin) capable of delaying the release of the drug until a predetermined time interval after ingestion. The following is prepared in such a manner that, after ingestion, there will be no release for a preset time interval (5-8 hours). Thus, if taken at bedtime it reaches optimal therapeutic blood levels at a time in the early morning when the events leading up to a vascular obstruction culminating in a heart attack or stroke are most commonly occurring after the drug is taken in the evening. The formulation comprises of an aspirin core together with a swelling agent and a frangible coating protecting aspirin from dissolution by gastrointestinal fluids having water soluble and insoluble properties.

- **Reservoir systems with soluble or eroding polymer coatings:**

Another class of reservoir-type multiparticulate pulsatile systems is based on soluble / erodible polymer coatings in which, barrier dissolves or erodes after a specific lag time followed by burst release of drug from the reservoir core. In general, for this kind of systems, the lag time prior to drug release can be controlled by the thickness of the coating layer. The basic principle employed in these systems is that of pH – sensitive polymers complimenting to their large increase in solubility at same point in the g.i.t. this sensitivity has been utilized to prevent release in the stomach affording complete release in intestine. However, since from these systems release mechanism is dissolution, a higher ratio of drug solubility relative to the dosing amount is essential for rapid release of drug after the lag period. Kao et al., 1997 studied the lag times delayed by the hydration of various thicknesses of polymer (Eudragit RS) films in an attempt to deliver drugs (Diltiazem hydrochloride) to various sites in the g.i.t. In a theoretical simulation, it was found that the lag time could be controlled by varying the thickness of the coated polymer, which was equivalent to the amount of the dry polymer in coating. The relationship between the lag time and the square of the amount of polymer coated, as well as that between the release rate at steady state and the inverse of the amount of polymer coated was well predicted.

Gazzaniga et al., 1994 developed a multi-unit system with a reservoir drug coated with a high viscosity polymer (HPMC 4000) and an outer enteric coating. The outer film protects thesystem from the stomach fluids and dissolves on entering the small intestine. HPMC layer delays the release of drug for 3-4 h when the system is transported through small intestine.

Pawar et al., 2007 developed a system comprised of multicoated multiparticulates for time controlled pulsatile release. One of the coating membranes is an enteric polymer and the second membrane barrier is a mixture of a water-insoluble polymer and an enteric polymer. An organic acid, such as fumaric acid, citric acid, succinic acid, tartaric acid, or malic acid, may be provided between the first and second membrane layers to provide for the time-separated pulses. The acids in between the membranes may delay the dissolution of the enteric polymer in the inner layer, thereby increasing the lag time as well as decreasing the rate of release of the active ingredient from the coated microparticulates. This type of system has been utilised for the weakly basic drugs (which shows pH – dependent solubility and hence insoluble at intestinal pH) because the system contains pH adjusters which maintains the local acidic environment within the system.

- **System with changed membrane permeability:**

Abundant pharmaceutical forms with delayed release for oral administration are available. The release of the drug must be controlled according to therapeutical rationale and the pharmacological properties of the active ingredient. In consequence, it is not always desirable the blood levels to be steady. On the contrary, in order to avoid any habituation and in order to limit the side effects aggravated by the active ingredient, it would be absolutely beneficial for the plasmatic rate to follow the metabolic rhythm and the specific needs of the patient during certain periods. For instance, in order to diminish the nocturnal symptoms or the symptoms upon awakening in the case of certain chronic diseases which are affected by the circadian biorhythms (ischemic heart disease, asthma and arthritis), the drugs should be administered in such a way that the desired therapeutical plasmatic level is reached only at the desired moment, i.e. during sleep or at the moment of awakening (Kalantzi et al., 2009). The release profile in this system depends on physico – chemical properties of drug and its interaction with the membrane.

Chen et al., 1996 designed osmotic multiparticulate delivery systems in which the drug released in divided doses over timed intervals throughout the day to produce pulsatile blood concentration curve with time. Each pellet contains a drug (Diltiazem) containing core, and a water soluble osmotic agent (NaCl) enclosed in a water permeable, water-insoluble polymer film. Incorporated into the polymer film is a hydrophobic, water insoluble agent which alters the permeability of the polymer film. The film coating of each population of pellets differs from the coating of every other population of pellets in the dosage form in the rate at which water passes through to the core and the rate at which drug diffuses out of the core. The osmotic agent dissolves in the water, causing the pellet to swell and regulating the rate of diffusion of drug into the environment of use. Sigmoidal release pattern is therapeutically beneficial for timed release and colonic drug delivery, and is observed in coated systems. A Sigmoidal release pattern obtained is based on the permeability and water uptake of polymers and influenced by the presence of different counter – ions in the release medium (Bodmeire et al., 1996). Narisawa *et. al.* (Narisawa et al., 1993; Narisawa et al., 1996) developed a system with such type of ion exchange. Eudragit RS 30D is reported to be a polymer of choice for this purpose. It typically contains positively polarized quaternary ammonium group in the polymer side chain, which is always accompanied by negative hydrochloride counter-ions. The ammonium group being hydrophilic it facilitates the interaction of polymer with water, thereby changing its permeability and allowing water to permeate the active core in a controlled manner. They found that a core of theophylline coated with Eudragit RS 30D showed very slow release in pure water but a significant increase in the release rate when immersed in an organic acid solution containing succinic, acetic, glutaric, tartaric, malic or citric acid. This is due to higher hydration of the film containing quaternary ammonium groups on interaction with the acids. (Parul B Patel 2011)

Future Prospects:

Recent information indicates a growing interest in colon as a site for chronotherapeutics, which seems to be quite promising as in certain disease states (which are affected by circadian Journal of Applied Pharmaceutical Science 01 (05); 2011: 59-63 biorhythms. From the technological point of view, multiparticulate systems seems to be more efficient than single unit dosage forms provides several all the advantages including greater flexibility and adaptability of multiparticulate systems, which gives researchers powerful new tools to optimize therapy. An increasing number of multiparticulate systems would possibly become commercially available in the near future.

- **Microspheres of anti-cancer drugs:** Cross-linked guar gum microspheres containing methotrexate were prepared and characterized for local release of drug in the colon for efficient treatment of colorectal cancer. In this method glutaraldehyde was used as a cross-linking agent and guar gum microspheres were prepared by emulsification method. From the results of in vitro and in vivo studies the methotrexate loaded cross-linked guar gum microspheres delivered most of the drug load (79%) to the colon, where as plain drug suspensions could deliver only 23% of their total dose to the target tissue. Colon specific microspheres of 5-fluorouracil were prepared and evaluated for the treatment of colon cancer. In this method core microspheres of alginate were prepared by modified emulsification method in liquid paraffin and by cross-linking with calcium chloride. The core microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach and small intestine. The results showed that this method had great potential in delivery of 5-fluorouracil to the colon region (Ziyaus et al. 2006).

CODES:

CODES are a unique colon targeted drug delivery system that was designed to avoid the inherent problems associated with pH or time dependent systems. It consists of core tablets coated with three layers of polymer coatings. The first coating is an acid soluble polymer (Eudragit) and outer layer is enteric with a HPMC barrier layer in between to prevent any possible interaction between the oppositively charged polymers. The core tablet is comprised of the active ingredients and one or more polysaccharides. The polysaccharides are degraded by enterobacteria to generate organic acid. During its transit through GIT, CODES remain intact in the stomach due to enteric protection, but the enteric barrier coating dissolves in the small intestine, where pH is is above 6. Because Eudragit-E starts to dissolves at pH 5; the inner Eudragit-E coating is only slightly permeable and swellable in small intestine. Upon entry into the colon, the bacteria enzymetically degrade the polysaccharide into organic acid. (M.k.chourasia 2003)

Evaluation of CDDS:

The drug release in the colonic region from different CDDS is evaluated by different methods of in vitro and in vivo release studies, which show the success rate of different designs of colon drug delivery systems. Depending upon the method of preparation different evaluation methods are proposed. A successful colon specific drug delivery system is one of that remains intact in the physiological environment of stomach and small intestine, but releases the drug in the colon.

In-vitroEvaluation:

Different in vitro methods are used to evaluate the colonic drug delivery systems. In in-vitro studies the ability of the coats/carriers to remain intact in the physiological environment of the stomach & small intestine is assessed by drug release studies in 0.1N HCl for two hours (mean gastric emptying time) and in pH 7.4 phosphate buffer for three hours (mean small intestine transit time) using USP dissolution apparatus. In case of micro flora activated system dosage form, the release rate of drug is tested in vitro by incubating in a buffer medium in the presence of either enzymes (e.g. pectinase, dextranase) or rat/guinea pig / rabbit caecal contents. The amount of drug released at different time intervals during the incubation is estimated to find out the degradation of the carrier.

In-vivoEvaluation:

Like other controlled release delivery systems, the successful development of the CDDS is ultimately determined by its ability to achieve release in colonic region thus exerts the intended therapeutic effect. When the system design is concerned & prototype formulation with acceptable in vitro characteristics is obtained, in vivo studies are usually conducted to evaluate the site specificity of drug release and to obtain relevant pharmacokinetic information of the delivery system. Although animal models have obvious advantages in assessing colon specific drug delivery systems, human subjects are increasingly utilized for evaluation of this type of delivery systems. The preferable animals to evaluate CDDS are rats, guinea pigs and dogs.

Animal models:

Different animal model are used for evaluating in vivo performance of colon specific drug delivery system. Guinea pigs were used to evaluate colon specific drug delivery from a glucoside prodrug of dexamethasone. Other animal model used for the in vivo evaluation of colon specific drug delivery system includes the rat and the pig.Technique for monitoring in vivo behavior of the colon specific drug delivery system in humans: A variety of technique like

- String technique
- Endoscopy
- Radiotelemetry
- Roentgenography and
- Gamma scintigraphy.

Were used for monitoring the in vivo behavior of a oral dosage forms.

String technique:

This technique was first applied by Gruber et. al. (1958) and Steinberg (1965). In these studies, a tablet was attached to the piece of string and the swallowed the tablet, leaving the free end of the string hanging from his mouth. At various time points, the tablet was withdrawn from the stomach by pulling out the string and physically examining the tablet for the sing of disintegration. In some studies, the tablets were recovered by inducing a vomiting reflex. The presence of foreign object, such as string in GI tract may alter its motility and physicochemical environment. The psychological stress and anxiety associated with this method also affect the motility of GI tract. (S.P.vyas n.d.)

Endoscope technique*:*

This technique has been employed by Hey et al, (1979). It is an optical technique in which a fibre scope (gastroscope) is used to directly monitor behavior of dosage form after ingestion. This method required administration of a mild sedative to facilitate the swallowing of endoscopic tube. The sedative its self may alter gastric emptying and GI motility. The psychological factor also contributes to the change in motility of the GI tract. (H.Hey 1982)

Radiotelemetry:

This technique involve the administration of capsule that consist of small pH probe interfaced with a miniature radio transmitter which is a capable of sending a signal indicating the pH of environment to non external antenna attached to the body of subject, so it is necessary to physically attach the dosage form to the capsule which in turn may affect the behavior of dosage form being studied. Furthermore dosage form must be contained significant amount of buffer salt, the release of which produces a change in gastrointestinal pH that is detected by the pH capsule indicating the change dosage form. This prevents the evaluation of commercially available product which does not contain buffer salts. (B.kinter 2003), (H.M.Brash 1976)

Roentgenography:

The inclusion of radio opaque material in to a solid dosage form enables it to be visualized by use of X-rays. By incorporating barium sulphate in to pharmaceutical dosage form, it is possible to follow the movement, location and integrity of dosage form after oral administration by placing the subject under a fluoroscope and taking the series of X-ray at various points. This method was first used by Losinsky & Diver (1933) and has been used by many subsequent workers. This technique use by Dew et al (1982) to evaluate the capsule dosage form coat with Eudragit to deliver a orally ingested drug to the colon using barium sulphate as radio opaque material the use of X-rays involves the exposing the subject to fairly high radiation dose as several photograph must be taken. Information can not be obtained on continuous basis. The radio opaque material, such as barium sulphate has high density and may not be good model. (W.H.Browers n.d.)

Gamma scintigraphy:

The most useful technique, to date, to evaluate the in vivo behaviour of dosage form in animals & humans is external scintigraphy or gamma scintigraphy. Work in this area began in 1970 through the modification of standard nuclear medicine method, to monitor the in vivo behaviour of dosage forms. Gamma scintigraphy requires the presence of gamma emitting radioactive isotopes in the dosage forms that can be detected in vivo by an external gamma camera. The dosage forms can be radiolabelled using conventional labeling or neutron activation methods.

Of all these methods available, gamma scintigraphy is the most widely used noninvasive technique for studying the in vivo behaviour of oral dosage form under the normal physiological condition. (H.M.Brash 1976) (Hebden J.M 1999) (Adkin D.A 1997)

- **Advantages:**

Gives a very little radiation exposure to the participating subject compared to Roentgenography (X-Ray)

a) Gives both qualitative & quantitative results, which is not possible with other techniques
b) Totally noninvasive method &
c) Allows for the in vivo evaluation of dosage forms under normal physiological conditions. A brief account of gamma scintigraphy including methodology & applications is given below.

- **Disadvantages:**

Inability to accurately quantify the activity in the small bowel because of its coiled nature, b) it is not possible to label all compounds /Drug of interest's) the gamma scintigraphy assembly is expensive d) require qualified personnel for operation.

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CHRONOPHARMACEUTICAL DRUG DELIVERY SYSTEM

Chavan Shankar, Shaikh Aamir

1.Introduction:

Over the last 30 years the pharmaceutical market has been demonstrated increasing preferably for controlled and targeted drug delivery system. Such systems have been focused on constant, variable; sustain drug release and/or targeting the therapeutic agent to a specific site/tissue/ organ. However, recently there are certain conditions for which such release pattern is not suitable. Such conditions that lead to the requirements of a time programmed therapeutic system, which capable of releasing drug after predetermined time delay and maintain constant drug levels throught the day. To introduce the concept of chronotherapeutics, it is important to define the following concepts (VIPUL P. PATEL n.d.)

Chronobiology:

Chronobiology is the science concerned with the biological mechanism of the diseases according to a time structure.“Chrono” pertains to time and “biology” pertains to the study, or science, of life.

Chronopharmacology:

Chronopharmacology is the science concerned with the variations in the pharmacological actions of various drugs over a period of time of the day.

Chronopharmacokinetics:

Chronopharmacokinetics involves study of temporal changes in drug absorption, distribution, metabolism and excretion. Pharmacokinetic parameters, which are conventionally considered to be constant in time, are influenced by different physiological functions displaying circadian rhythm. Circadian changes in gastric acid secretion, gastrointestinal motility, gastrointestinal blood flow, drug protein binding, liver enzyme activity, renal blood flow and urinary pH can play role in time dependent variation of drug plasma concentrations.

Chronotherapy:

Co-ordination of biological rhythms and medical treatment is called chronotherapy.

Chronotherapeutics:

Chronotherapeutics is the discipline concerned with the delivery of drugs according to inherent activities of a disease over a certain period of time.

It is becoming increasingly more evident that the specific time that patients take their medication may be even more significant than was recognized in the past. (VIPUL P. PATEL n.d.)

2. Biological Rhythms:

1. Ultradian Rhythms:

Oscillations of shorter duration are termed Ultradian Rhythms (more than one cycle per 24 h). E.g.90 minutes sleep cycle. (VIPUL P. PATEL n.d.)

2. Infradian Rhythms:

Oscillations that are longer than 24 hours are termed as Infradian Rhythms (less than one cycle per 24hours). E.g. Monthly Menstruation. (VIPUL P. PATEL n.d.)

3. Circadian rhythms:

Circadian rhythms are self-sustaining, endogenous oscillations that occur with a periodicity of about 24 Hours. Interestingly, the term circadian is derived from the Latin circa which means “about” and dies which can be defined as “a day”. Normally, circadian rhythms are synchronized according to internal biologic clocks related to the sleep-wake cycle. (VIPUL P. PATEL n.d.)

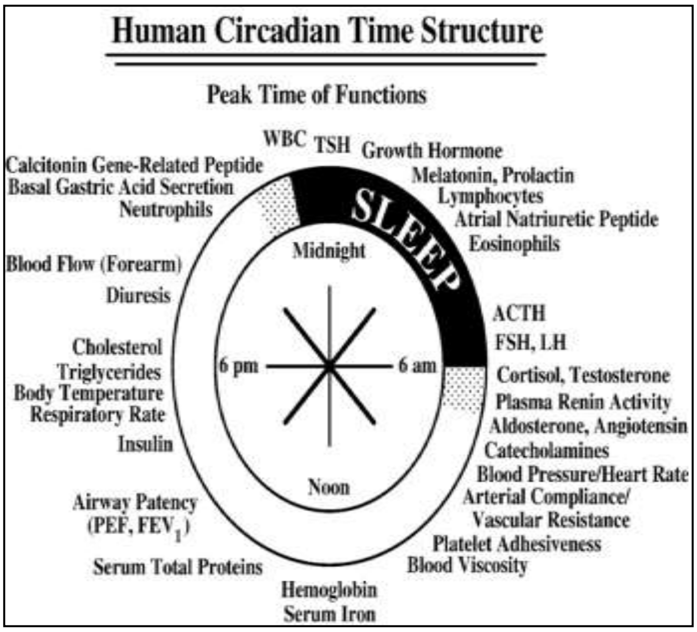


Fig.1: Human Circadian time structure (P. Rathee 2011)

3. Diseases & Chronotherapeutics:

The potential benefits of chronotherapeutics have been demonstrated in the management of a number of diseases. In particular there is a great deal of interest in how chronotherapy can particularly benefit patients suffering from allergic rhinitis, rheumatoid arthritis and related disorders, asthma, cancer, cardiovascular diseases, and peptic ulcer disease. (VIPUL P. PATEL n.d.)

Cardiovascular Diseases:

Cardiovascular diseases such as hypertension and angina, or chest pain, also follow a definite circadian rhythm. (VIPUL P. PATEL n.d.)

Hypertension:

Heart rate and blood pressure are increased in the early morning hours (morning or A.M. surge). The blood pressure declines form mid afternoon and is minimum at midnight. In most hypertensive patients, there is a rather marked rise in blood pressure upon awakening that is called the morning or "a.m." Systolic blood pressure rises approximately 3mm Hg/hour for the first 4-6 hours post-awakening, while the rate of rise of diastolic blood pressure is approximately 2mm Hg/hour. (VIPUL P. PATEL n.d.)

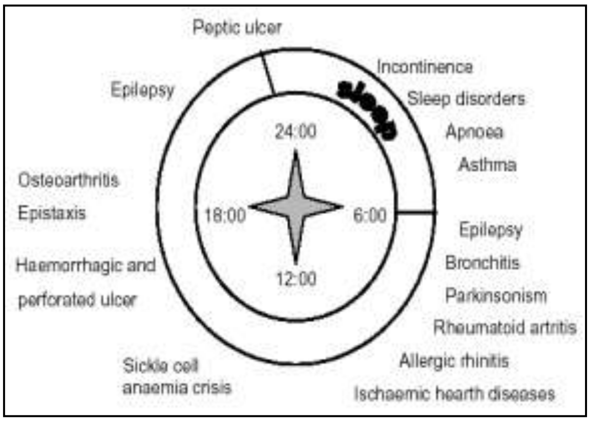


Fig.2: Diseases displaying circadian rhythm (J Sajan 2009)

Myocardial Infarction:

Onset of myocardial infarction has been shown to be more frequent in the morning with 34% events occurring between 6 A.M. and noon. Acute cardiac arrest and transient myocardial ischemia shows an increased frequency in morning. The causes for these findings have been suggested to be release of catecholamine, cortisol increase in the platelet aggregation and vascular tone. ACE inhibitors are more effective when administered during night. Atenolol, Nifedipine and amolodipine are more effective when administered at night. (VIPUL P. PATEL n.d.)

Neoplastics:

Antineoplastic drugs cause cytotoxic effects on healthy and diseased tissues. As would be expected, the biological rhythms of both healthy and tumor cells may influence the susceptibility of normal and malignant cells to these agents [14]. It has been demonstrated that "susceptibility rhythms" to drugs may differ between healthy tissue and cancerous tissue. Therefore, the "correct" timing of drug treatment may reduce host toxicity, increase maximum drug tolerance, and ultimately result in better tumor management. The pharmacologic and pharmacokinetic properties of the drug, rhythmic changes in DNA and RNA synthesis, RNA translational activity and mitotic activity may influence tumor cell susceptibility [3]. It appears that the timing of drug administration in the treatment of cancer can have a significant impact upon treatment success. (J Sajan 2009)

Peptic ulcer disease:

Because of maximal acid secretion, peptic ulcer disease pain, and perforation of gastric and duodenal ulcers are more common at night, administration of drugs at bedtime is more effective. Nocturnal administration not only reduces acid secretion more effectively but also promotes ulcer healing and reduces ulcer recurrence. Bedtime H2-receptor blockade is one such regimen. (VIPUL P. PATEL n.d.)

Bronchial asthma:

Asthma is a chronic inflammatory disease of the airways, characterized by hyper responsiveness to a variety stimuli. The role of circadian rhythms in the pathogenesis and treatment of asthma indicates that airway resistance increases progressively at night in asthmatic patients. Circadian changes are seen in normal lung function, which reaches a low point in the early morning hours. The worsening of asthma at night, commonly referred to as nocturnal asthma (NA). A drug delivery system administered at bedtime but releasing drug during morning hours would be ideal in this case. (VIPUL P. PATEL n.d.)

Chronological behavior	Drugs used	Diseases
Acid secretion is high in the Afternoon and at night	H2 blockers	Peptic ulcer
Precipitation of attacks during night or at early morning	β2 agonist, Antihistamines	Asthma
BP is at its lowest during the sleep cycle and rises steeply during the early morning	Nitroglycerin, calcium channel blocker, ACE inhibitors	Cardiovascular diseases
Pain in the morning and more pain at night	NSAIDs, Glucocorticoids	Arthritis
Increase in the blood sugar level after meal	Sulfonylurea, Insulin	Diabetes mellitus
Cholesterol synthesis is generally higher during night than day time	HMG CoA reductase Inhibitors	Hypercholesterolemia

Table 1. Diseases requiring pulsatile delivery

Chronopharmaceutical Drug Delivery system:

Pulsatile system is amongst one of them and gaining a lot of interest as it is increasing patient compliance by means of providing time- and site-specific drug delivery system, thus providing special and temporal delivery. Pulsed or pulsatile drug release is defined as the rapid and transient release of a certain amount of drug molecules within a short time-period immediately after a predetermined off-release period3. Recent studies show that diseased have predictable cyclic rhythms and the timing of medication regimens can improve outcome in selected chronic conditions. Numerous studies conducted, suggest that pharmacokinetics, drug efficacy and side effects can be modified by following therapy matching the biological rhythm. Specificity in delivering higher amount of drug in a burst at circadian timings correlated with specific pathological disorder is a key factor to achieve maximum drug effect. “Chronopharmaceutics” consists of two words chronobiology and pharmaceutics. Chronobiology is the study of biological rhythms and their mechanisms. (VIPUL P. PATEL n.d.)

3.Necessities of chronopharmaceutical DDS:

1. First pass metabolism:

Some drugs, such as beta blockers and salicylamide undergo extensive first pass metabolism and require fast drug input to saturate metabolizing enzymes in order to minimize pre-systemic metabolism. Thus, a constant/sustained oral method of delivery would result in reduced oral bioavailability. (GANESH RASVE 2011)

2. Biological tolerance:

Drug plasma profiles are often accompanied by a decline in the pharmacotherapeutic effect of the drug, e.g., biological tolerance of transdermal nitroglycerin, salbutamol sulphate. (GANESH RASVE 2011)

3. Special chronopharmacological needs:

Circadian rhythms in certain physiological functions are well established. It has been recognized that many symptoms and onset of disease occur during specific time periods of the 24 hour day, e.g., asthma and angina pectoris attacks are most frequently in the morning hours. (GANESH RASVE 2011)

4. Local therapeutic need:

For the treatment of local disorders such as inflammatory bowel disease, the delivery of compounds to the site of inflammation with no loss due to absorption in the small intestine is highly desirable to achieve the therapeutic effect and to minimize side effects. (GANESH RASVE 2011)

5. Gastric irritation or drug instability in gastric fluid:
Protection from gastric environment is essential for the drugs that undergo degradation in gastric acidic medium (eg, peptide drugs), irritate the gastric mucosa (NSAIDS) or induce nausea and vomiting. (GANESH RASVE 2011)

6. Drug absorption differences in various gastro-intestinal segments: In general, drug absorption is moderately slow in the stomach, rapid in the small intestine, and sharply declining in the large intestine. Compensation for changing absorption characteristics in the gastrointestinal tract may be important for some drugs. For example, it is rational for a delivery system to pump out the drug much faster when the system reaches the distal segment of the intestine, to avoid the entombment of the drug in the feces (P. Rathee 2011)

MERITS:

1. Predictable, reproducible and short gastric residence time
2. Less inter- and intra-subject variability
3. Improve bioavailability
4. Limited risk of local irritation
5. No risk of dose dumping
6. Flexibility in design
7. Improve stability. (GANESH RASVE 2011)

DEMERITS:

1. Lack of manufacturing reproducibility and efficacy
2. Large number of process variables
3. Batch manufacturing process
4. Higher cost of production
5. Trained/skilled personal needed for manufacturing. (GANESH RASVE 2011)

4. Dosage forms:

Types of dosage forms that can be designed:-

Compression coated/press coated tablets:

These are timed release formulations, simple to manufacture, comprised of an inner core that contains an active pharmaceutical ingredient and excipients surrounded by an outer layer that dissolves or disintegrates slowly to produce the lag time. The core is placed between two layers of polymer and directly compressed by flat punches of tablet machine. Surrounding polymeric layers protect the drug from release before the desired lag time, hence effective delivery in chronotherapy as it allows the drug release at the point in circadian cycle when clinical signs develop and increase. Drugs that treat cardiovascular disease (nifedipine, nitrendipine, amlodipine, diltiazem etc) and asthma (theophylline, budesonide) had been attempted to formulate such dosage forms. Swada *et al.* 2003 prepared timed release compression coated tablets of nifedipine for chronotherapy of angina and compared it *in vitro-in vivo* release profile with sustained release formulation. (Lida E. Kalantzi 2009)

Core in cup tablets:

It is a novel oral pulsatile release drug delivery system based on a core-in-cup dry coated tablet, where the core tablet surrounded on the bottom and circumference wall with inactive material. The system consists of three different parts, a core tablet, containing active ingredient, an impermeable outer shell and a top cover layer-barrier of a soluble polymer. The impermeable coating cup consisted of cellulose acetate propionate and the top cover layer of hydrophilic swellable materials such as polyethylene oxide, sodium alginate or sodium carboxy methyl cellulose. The system releases the drug after a certain lag time generally due to the erosion of top cover layer. The quantity of material, its characteristics (viscosity, swelling, gel layer thickness) and the drug solubility was found to modify lag time and drug release. The lag time increases when quantity of top layer increases, whereas drug release decreases. (Lida E. Kalantzi 2009)

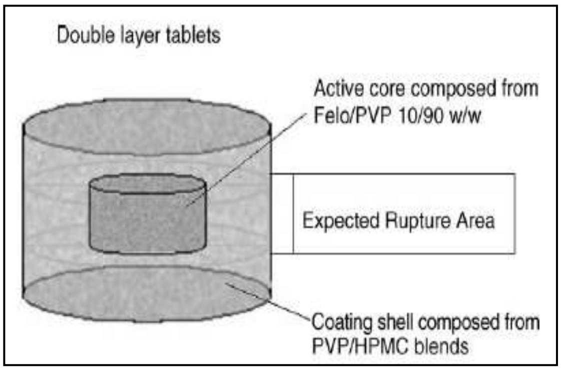

Double layer tablets
Active core composed from Felo/PVP 10/90 w/w
Expected Rupture Area
Coating shell composed from PVP/HPMC blends

Fig. 3: Design of the press coated tablet composed of an active FELODIPINE/PVP core and an inactive PVP/HPMC coating layer. (Lida E. Kalantzi 2009)

Pulsincap systems:

As discussed previously that these are the well designed pulsatile release drug delivery systems capable of releasing drug at a pre determined time. Drug formulation is contained within the insoluble capsule body which is sealed by means of a hydrogel plug. On oral administration the water soluble capsule cap dissolves in the gastric juices and hydrogel plug swells. At a controlled and predetermined time point after the ingestion, the swollen plug is ejected from the pulsincap dosage form after which the encapsulated dosage formulation is then released. To simplify this technology, the hydrogel plug has been replaced by an erodible tablet, which has a tight fit in capsule to prevent the entry of fluid. During the release process it erodes away from the mouth of capsule. The effect of various parameters such as type and weight of swellable polymer, type of hydrophilic polymers used in erodible tablet formulation and erodible tablet weight was investigated in order to characterize the lag time and drug release profiles (Lida E. Kalantzi 2009).

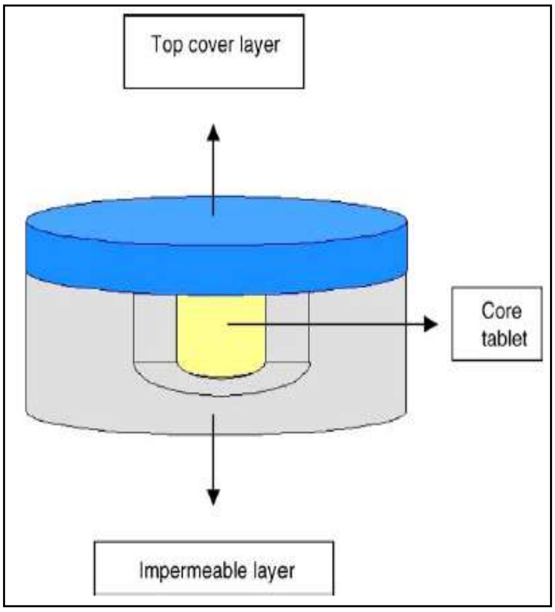


Fig. 4: Schematic representation of "core in cup tablet" as a pulsatile drug delivery system (Lida E. Kalantzi 2009)

The pulsatile capsule is designed for two drug doses. First is placed into the capsule cap while the second dose is released from an insoluble capsule body.lag time is determined by an osmotic system which presses an insoluble plug out of the capsule body.

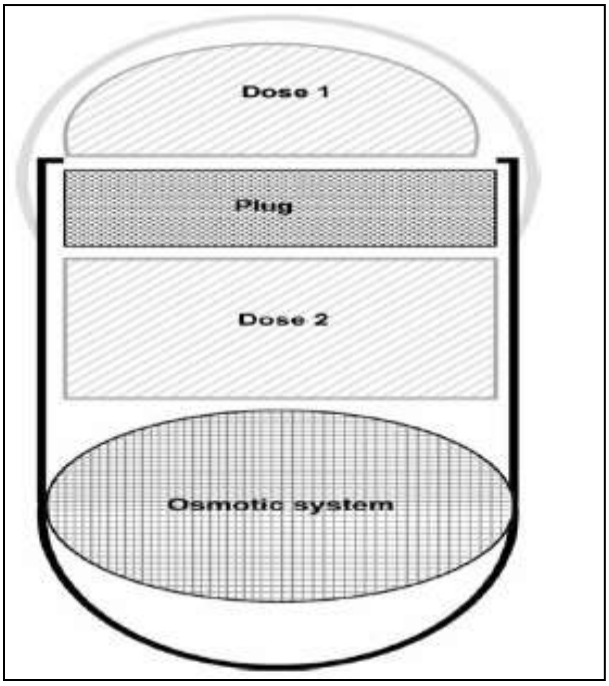


Fig. 5: Double coated hard gelatin capsules and double coated tablets (Lida E. Kalantzi 2009)

These are time controlled rupturable pulsatile drug delivery systems either in form of hard gelatin capsules tablets. The capsules are filled with active pharmaceutical ingredient either for single pulse or multi-pulse release (in form of multiparticulates) and coated with a swelling layer followed by an external water insoluble semipermeable polymeric coating. Upon water ingress the swelling layer swells to attain a threshold hydrodynamic pressure required to rupture the outer coating and allowing the release of contents in surrounding medium. The time required by swelling layer to rupture outer coating surves the purpose of desired lag time required in chrono therapy of disease. The tablets are manufactured and coated on the same principle as that of double coated gelatin capsules (Lida E. Kalantzi 2009)

Pulsatile release muliparticulate systems:

These systems have been developed on the basis of various approaches of designing pulsatile drug delivery system discussed later (like time controlled, stimuli induced or externally regulated pulsatile drug delivery systems).these can be developed in various types of dosage forms like:

i. Pellets

ii. Granules

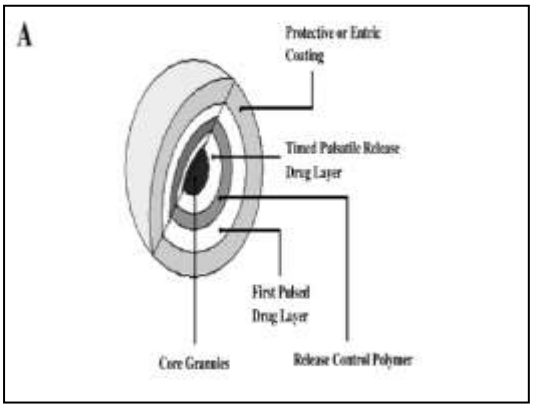
iii. Microspheres

iv. Beads

v. Nanoparticles

vi. Microsponges

In recent pharmaceutical applications involving pulsatile drug delivery, multiparticulate dosage forms are gaining much favour over single unit dosage forms. The potential benefits include increased bioavailability, predictable, reproducible, and generally short gastric residence time; no risk of dose dumping; reduced risk of local irritation; and flexibility to blend pellets with different compositions or release patterns. Because of their smaller particle size, these systems are capable of passing through gastrointestinal tract easily, leading to less inter- and intra-subject variability. A no. of multiparticulate pulsatile drug delivery systems have been developed for chronotherapy. For instance, colonic delivery of theophylline in form of microspheres and coated pellets for nocturnal asthma, formulation of indomethacin, ibuprofen, flurbiprofen, meloxicam, aceclofenac, diclophenac pellets and microspheres for chronotherapy of rheumatoid arthritis and floating beads of alginates encapsulating the active drug component in core, have been attempted to deliver many of the drugs which are absorbed in upper gastrointestinal tract. Numerous advanced technologies have been developed in designing of pulsatile release multiparticulate dosage forms and many of them are FDA approved and being marketed. (Lida E. Kalantzi 2009)


A
Protective or Entric Coating
Timed Pulsatile Release Drug Layer
First Pulsed Drug Layer
Core Granules
Release Control Polymer

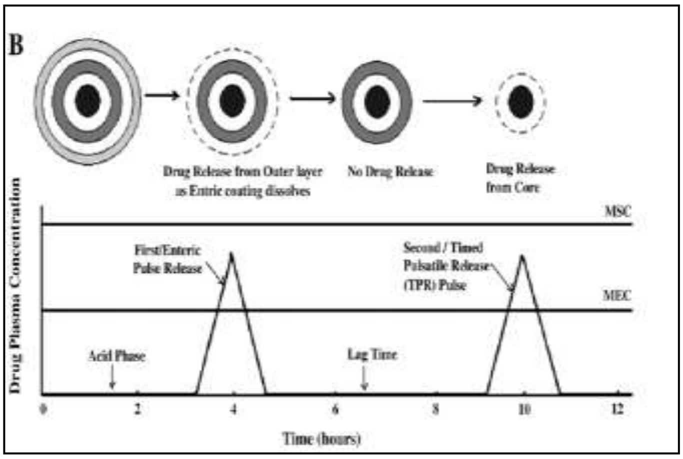


Fig. 6: Hypothetical design and plasma drug profile of a multiparticulate pulsatile system. (A) Design of a pellet with multiple coatings, and (B) Predicted bi-modal plasma concentration profile.

Chronomodulating infusion pumps:

Externally and internally controlled systems across a range of technologies including pre-programmed systems, as well as systems that are sensitive to modulated enzymatic or hydrolytic degradation, pH, magnetic fields, ultrasound, electric fields, temperature, light and mechanical stimulation have been reviewed in detail elsewhere. To our knowledge infusion pumps on the market that have been referred to as chronomodulating for drug delivery application include the Melodie, programmable Synchromed, Panomat V5 infusion, and the Rhythmic pumps. The portable pumps are usually characterized by a light weight (300-500 g) for easy portability and precision in drug delivery. For example portable programmable multi-channel pumps allowed demonstration of the clinical relevance of the chronotherapy principle in a sufficiently large patient population. Specifically, a clinical phase III trial involving several patients with metastatic gastrointestinal malignancies compared a flat versus the chronomodulated three-drug regimen, and demonstrated large, simultaneous improvements in both tolerability and response rates in patients with metastatic colorectal cancer receiving chronotherapy. In case of insulin therapy, implantable infusion pumps containing a reservoir of insulin may be surgically placed within the subcutaneous tissue of the abdomen in the left upper or lower quadrant (above or below the belt). A catheter leads from the pump through the muscle layers into the peritoneal cavity, where it floats freely, and insulin delivery is by the intraperitoneal route. The insulin reservoir is refilled once a month or every 3 months at a physician's office by inserting a needle through the skin into the pump (a local anesthetic is first used). Doses adjustments are made by the patient (within ranges established by the physician) using radiotelemetry and an electronic device that is held over the pump. Their advantages include the fact that the peritoneum provides a large, well-vascularized surface area, and absorption is faster by this route than after subcutaneous injection (better insulin gradient), improved glycemic control and a reduction in the frequency of hypoglycemic episodes. Possible drawbacks of this approach include eventual formation of fibrous tissue pocket and local skin erosion. Catheter blockade which can reduce insulin delivery, are the most common problems with implantable pumps. However, these pumps have been effectively used in the chronotherapy of several diseases such as cancer and diabetes (Gazzaniga A 1994)

Controlled-release microchip:

An alternative method to achieve pulsatile or chronopharmaceutical drug release involves using microfabrication technology. Santini *et al.* reported a solid-state silicon microchip that can provide controlled release of single or multiple chemical substances on demand. The release mechanism was based on the electrochemical dissolution of thin anode membranes covering microreservoirs filled with chemicals in solid, liquid or gel form. (Gazzaniga A 1994)

5. Approaches For Chronopharmaceutical DDS:

Various approaches of pulsatile drug:

Pulsatile drug delivery system can be broadly classified into three classes. (GANESH RASVE 2011)

I. Time controlled pulsatile drug delivery
II. Stimuli induced pulsatile drug delivery
III. Externally regulated pulsatile drug delivery
IV. Multipaticulate system

I. Time controlled pulsatile drug delivery:

A. Single unit pulsatile systems
1. Capsule based systems e.g. Pulisincap system
2. Capsular system based on Osmosis
    a. 'PORT' System
    b. System based on expandable orifice
    c. Delivery by series of stops.
    d. Pulsatile delivery by solubility modulation

3. Pulsatile system with Erodible or soluble barrier coatings.
    a. The chronotropic system
    b. 'TIME CLOCK' System
    c. Compressed tablets
    d. Multilayered Tablets

4. Pulsatile system with rupturable coating

B. Multiparticulate / Multiple unit systems:
1. Pulsatic system with rupturable coating
E.g. Time –controlled Explosion system (TCES)
2. Osmotic based rupturable coating system
E.g. Permeability controlled system
3. Pulsatile delivery by change in membrane permeability
E.g. Sigmoidal release system.

II. Stimuli induced pulsatile drug delivery:

A. Temperature–induced pulsatile release
1) Thermoresponsive hydrogel systems:
2) Thermoresponsive polymeric micelle systems:
B. Chemical stimuli-induced pulsatile release
1) Glucose-responsive insulin release devices
2) Inflammation-induced pulsatile release
3) Drug release from intelligent gels responding to antibody concentration.
4) pH sensitive drug delivery system

III. Externally regulated pulsatile drug delivery:

A. Magnetic induces release:
B. Ultrasound induces release:
C. Electric field induces release
D. Light induces release:

IV. Multipaticulate system:

I. Time controlled pulsatile drug delivery:

A. Single unit pulsatile systems

1. Capsule based systems:

Single-unit systems are mostly developed in capsule form. The lag time is controlled by a plug, which gets pushed away by swelling or erosion, and the drug is released as a "Pulse" from the insoluble capsule body. (VIPUL P. PATEL1 n.d.)

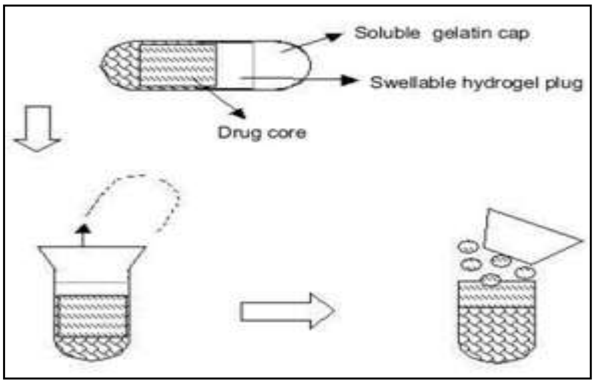


Fig 7: Design of Pulsincap system (S. R. Tajane (2012), Vol. 3, Issue 02)

The lag time can be controlled by manipulating the dimension and the position of the plug.

Polymers used for designing of the hydrogel plug

1) Insoluble but permeable and swellable polymers (e.g., polymethacrylates)

2) Erodible compressed polymers (e.g., hydroxypropylmethyl cellulose, polyvinyl alcohol, Polyethylene oxide)

3) Congealed melted polymers (e.g., saturated polyglycolated glycerides, glyceryl monooleate)

4) Enzymatically controlled erodible polymer (e.g., pectin).

The preparation and invitro release of tetramethylpyrazine phosphate pulsincap capsule has been reported. It was prepared by sealing the drug tablet and fillers inside an impermeable capsule body with erodible plug. To meet the chronotherapeutic requirements, a suitable lag time can be achieved by adjusting the content of gel-forming polymer (HPMC) and the erodible plug weight. (VIPUL P. PATEL1 n.d.)

2. Capsular system based on Osmosis

a. ‘PORT’ System:

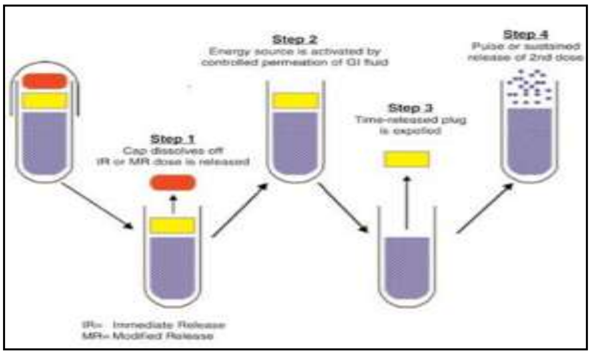

Step 2
Energy source is activated by controlled permeation of GI fluid
Step 4
Pulse or sustained release of 2nd dose
Step 3
Time-released plug is expelled
Step 1
Cap dissolves off IR or MR dose is released
IR= Immediate Release
MR= Modified Release

Fig 8: Drug release mechanism from PORT system (J. R Reddy 2009).

The Port system fig. (8) Was developed by Therapeutic system research laboratory Ann Arbor, Michigan, USA, and consists of a capsule coated with a semipermeable membrane. Inside the capsule was an insoluble plug consisting of osmotically active agent and the drug formulation. When this capsule came in contact with the dissolution fluid, the semipermeable membrane allowed the entry of water, which caused the pressure to develop and the insoluble plug expelled after a lag time. Such a system was utilized to deliver methylphenidate used in the treatment of attention deficit hyperactivity disorder as the pulsatile port system. This system avoided second time dosing, which was beneficial for school children during daytime. (VIPUL P. PATEL1 n.d.)

b. System based on expandable orifice: To deliver the drug in liquid form, an osmotically driven capsular system was developed in which the liquid drug is absorbed into highly porous particles, which release the drug through an orifice of a semipermeable capsule supported by an expanding osmotic layer after the barrier layer is dissolved. The orifice is small enough so that when the elastic wall relaxes, the flow of the drug through the orifice essentially stops, but when the elastic wall is distended beyond threshold value, the orifice expands sufficiently to allow drug release at a required rate. E.g. Elastomers, such as styrene-butadiene copolymer have been suggested. (VIPUL P. PATEL1 n.d.)

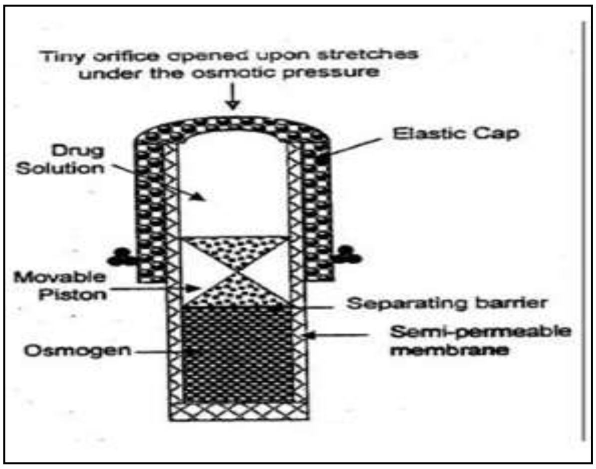


Fig.9: System based on expandable orifice (VIPUL P. PATEL n.d.)

c. Delivery by series of stops:

This system is described for implantable capsules. The capsule contains a drug and a water absorptive osmotic engine that are placed in compartments separated by a movable partition. The pulsatile delivery is achieved by a series of stops along the inner wall of the capsule. These stops obstruct the movement of the partition but are overcome in succession as the osmotic pressure rises above a threshold level. (VIPUL P. PATEL1 n.d.)

d. Pulsatile delivery by solubility modulation:

Such systems contain a solubility modulator for pulsed delivery of variety of drugs. The system was especially developed for delivery of salbutamol sulphate. The compositions contains the drug (salbutamol sulphate) and a modulating agent (sodium chloride). The amount of NaCl was such that it was less than the amount needed to maintain saturation in a fluid that enters the osmotic device. The pulsed delivery is based on drug solubility. Salbutamol has solubility of 275mg/ml in water and 16 mg/ml in saturated solution of NaCl, while NaCl has solubility of 321 mg/ml in water, and its saturation solubility is 320 mg/ml. (VIPUL P. PATEL1 n.d.)

3. Pulsatile system with Erodble or soluble barrier coatings:

Most of the pulsatile drug delivery systems are reservoir devices coated with a barrier layer. This barrier erodes or dissolves after a specific lag period, and the drug is subsequently released rapidly from reservoir core. The lag time depends on the thickness of the coating layer. (VIPUL P. PATEL1 n.d.)

a. The chronotropic system:

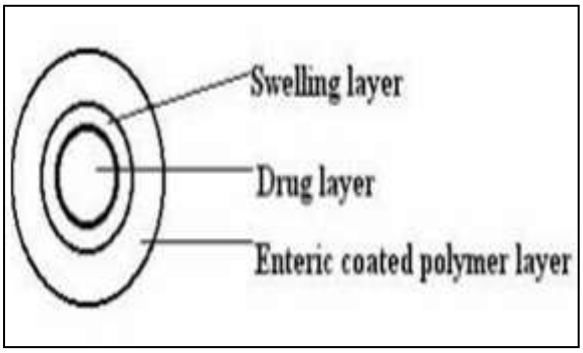


Fig.10: The chronotropic system (S. R. Tajane (2012), Vol. 3, Issue 02)

The Chronotropic® system consists of a drug-containing core coated by hydrophilic swellable hydroxypropylmethyl cellulose (HPMC), which is responsible for a lag phase in the onset of release. In addition, through the application of an outer gastric-resistant enteric film, the variability in gastric emptying time can be overcome, and a colon-specific release can be obtained, relying on the relative reproducibility of small intestinal transit time. The lag time is controlled by the thickness and the viscosity grades of HPMC. Both in-vitro and in-vivo lag times correlate well with the applied amount of the hydrophilic retarding polymer. The system is suitable for both tablets and capsules. (VIPUL P. PATEL1 n.d.)

b. 'TIME CLOCK' System:

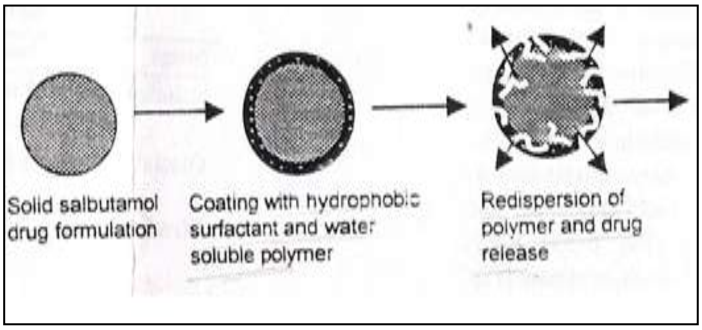

Solid salbutamol drug formulation
Coating with hydrophobic surfactant and water soluble polymer
Redispersion of polymer and drug release

Fig.11: 'TIME CLOCK' System (VIPUL P. PATEL n.d.)

The lag time could be controlled by varying the thickness of the film. After the lag time, i.e., the time required for rehydration, the core immediately releases the drug. This system has shown reproducible results in vitro and in vivo. The effect of low calorie and high calorie meal on the lag time was studied using gamma scintigraphy. The mean lag time of drug release was 345 and 333 min respectively. (VIPUL P. PATEL1 n.d.)

c. Compressed Tablets:

Compression coating can involve direct compression of both the core and the coat, obviating needs for separate coating process and use of coating solutions. The outer tablet of the compression-coated tablet provides the initial dose, rapidly disintegrating in the stomach and the inner layer is formulated with components that are insoluble in gastric media but are released in the intestinal environment. Materials such as hydrophilic cellulose derivates can be used. Compression is easy on laboratory scale. The major drawbacks of the technique are that relatively large amounts of coating materials are needed and it is difficult to position the cores correctly.

Press-coated pulsatile drug delivery systems:

1. Press-coated pulsatile drug delivery systems can be used to protect hygroscopic, light-sensitive, oxygenlabile or acid-labile drugs.
2. Press-coated pulsatile drug delivery systems are relatively simple and cheap.
3. These systems can involve direct compression of both the core and the coat.
4. Materials Such as hydrophobic, hydrophilic can be used in press-coated pulsatile drug delivery system.
5. Press-coated pulsatile drug delivery systems involve compression which is easy on laboratory scale.
6. Press-coated pulsatile formulations relese drug after "lag-time".
7. Press-coated pulsatile drug delivery formulations can be used to separate incompatible drugs from each other or to achieve sustained release. (VIPUL P. PATEL1 n.d.)

d. Multilayered Tablets:

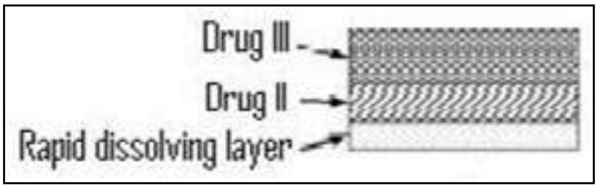


Fig.12: Multilayered Tablet (VIPUL P. PATEL n.d.)

A release pattern with two pulses was obtained from a threelayered tablet containing two drug containing layers separated by a drug-free gellable polymeric barrier layer. (VIPUL P. PATEL1 n.d.)

4. Pulsatile system with rupturable coating: These systems depend on the disintegration of the coating for the release of drug. The pressure necessary for the rupture of the coating can be achieved by the effervescent excipients, swelling agents, or osmotic pressure. An effervescent mixture of citric acid and sodium bicarbonate was incorporated in a tablet core coated with ethyl cellulose. The carbon dioxide developed after penetration of water into the core resulted in a pulsatile release of drug after rupture of the coating. The release may depend on the mechanical properties of the coating layer.

B. Multiparticulate / Multiple unit systems:

1. Pulsatile system based on rupturable coating: E.g. Time –controlled Explosion system (TCES):

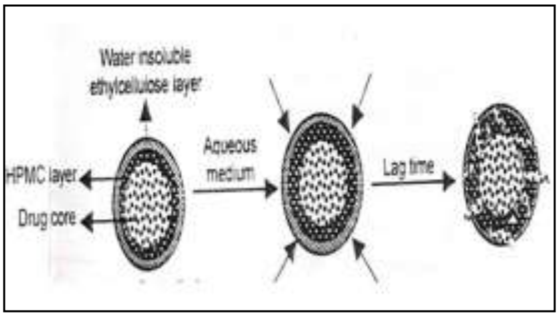


Fig.13: Time –controlled Explosion system (TCES) (VIPUL P. PATEL n.d.)

This is a multiparticulate system in which drug is coated on non-pareil sugar seeds followed by a swellable layer and an insoluble top layer. The swelling agents used include Superdisintegrants like sodium carboxymethyl cellulose, sodium starch glycollate, L hydroxypropyl cellulose. Polymers like polyvinyl acetate, polyacrylic acid, polyethylene glycol, etc. (VIPUL P. PATEL1 n.d.)

2. Osmotic based rupturable coating system: This system is based on a combination of osmotic and swelling effects. The core containing the drug, a low bulk density solid and/or liquid lipid material (eg, mineral oil) and a disintegrant was prepared. This core was then coated with cellulose acetate. Upon immersion in aqueous medium, water penetrates the core displacing lipid material. After the depletion of lipid material, internal pressure increases until a critical stress is reached, which results in rupture of coating. (Alessandra Maroni 2005)

3. Pulsatile delivery by change in membrane permeability:

The permeability and water uptake of acrylic polymers with quaternary ammonium groups can be influenced by the presence of different counter-ions in the medium. 48 Several delivery systems based on this ion exchange have been developed. Eudragit RS 30D is reported to be a polymer of choice for this purpose. It typically contains positively polarized quaternary ammonium group in the polymer side chain, which is always accompanied by negative hydrochloride counter-ions. The ammonium group being hydrophilic facilitates the interaction of polymer with water, thereby changing its permeability and allowing water to permeate the active core in a controlled manner. This property is essential to achieve a precisely defined lag time. (VIPUL P. PATEL1 n.d.)

4. Time controlled, low density floating pulsatile systems

As the name suggests these systems are comprised of low density floating pulsatile dosage forms, reside in stomach only and not affected by variation in gastric ph, local environment or gastric emptying rate. These dosage forms may be either single unit (floating tablets) or multiparticulates (beads, pellets, granules, microspheres) with capability of gastro-retention. These are specifically advantageous for drugs either absorbed from the stomach or requiring local delivery in stomach. Polysaccharides are widely used in oral delivery systems because of simplicity to obtain the desired drug delivery system and drug release profile, by the control of cross linking, insolubility of cross linked beads in gastric environment and broad regulatory acceptance. Badave et al developed hollow calcium pectinate beads for floating pulsatile release of diclofenac sodium intended for chronotherapy.

A multiparticulate floating pulsatile drug delivery system was developed using porous calcium silicate (Fluorite RE) and sodium alginate for time and site specific drug release of meloxicam for chronopharmacotherapy of rheumatoid arthritis. Meloxicam was adsorbed on the Fluorite RE by fast evaporation of solvent from drug solution containing dispersed Fluorite. Drug adsorbed fluorite powder was used to prepare calcium alginate beads by ionotropic gelation method. The floating time for this system was controlled by density of beads and hydrophobic character of drug. To overcome limitations of various approaches for imparting buoyancy hollow/porous calcium pectinate beads were prepared by simple process of acid base reaction during ionotropic cross linking. The floating beads provide two-phase release pattern with initial lag time during floating in acidic medium followed by rapid pulse release in phosphate buffer. These drug delivery systems show distinct behavior from other approaches in chronotherapy with desired low drug release in acidic medium, reduced time consumption due to single step process and also overcame the limitations of process variable caused by multiple formulation steps. (Mayank Nagar 2010)

II. Stimuli-induced pulsatile release:

A. Temperature–induced pulsatile release:

This deviation sometimes can act as a stimulus that triggers the release of therapeutic agents from several temperatureresponsive drug delivery systems for diseases accompanying fever. The temperature induced pulsatile/triggered drug delivery systems utilize various polymer properties, including the thermally reversible coil/globule transition of polymer molecules, swelling change of networks, glass transition and crystalline melting. (GANESH RASVE 2011)

1) Thermoresponsive hydrogel systems:

Thermo-responsive hydrogel systems employ hydrogels which undergo reversible volume changes in response to changes in temperature. These gels shrink at a transition temperature that is referred to the lower critical solution temperature (LCST) of the linear polymer. Thermo-sensitive hydrosensitive hydrogels have a certain chemical attraction for water, and therefore they absorb water and swell at temperatures below the transition temperature whereas they shrink or deswell at temperatures above the transition temperature by expelling water. Thermally responsive hydrogels and membranes have been extensively exploited as platforms for the pulsatile drug delivery. (GANESH RASVE 2011)

2) Thermoresponsive polymeric micelle systems*:*

In this type, the gel system tightly stores targeted drug in the micelles and rapidly releases controlled amount of the drug by switching on–off of external stimuli such as temperature or infrared laser beam. Jianxiang Zhang, et al synthesized thermally responsive amphiphilic poly(N isopropylacrylamide) (PNIPAm)-graftedpolyphosphazene (PNIPAm-g- PPP) by stepwise cosubstitution of chlorine atoms on polymer backbones with amino-terminated NIPAm oligomers and ethyl glycinate (GlyEt). Diflunisal (DIF)-loaded micelles were prepared by dialysis method. In vitro release test at various temperatures was also performed to study the effect of temperature on the drug release profiles. (GANESH RASVE 2011)

B. Chemical stimuli-induced pulsatile release:

1) Glucose-responsive insulin release devices:

A decrease in or the absence of insulin secretion from pancreatic islets is the cause of diabetes mellitus. Diabetes mellitus patients suffer long term from a gradual decline in the efficiency of various organs, such as the occasional loss of eyesight. Several systems have already been developed which are able to respond to glucose concentration changes. Glucose oxidase (GOD) catalyzes glucose oxidation. Utilizing this reaction, Ishihara et al. prepared two types of gel membrane systems to regulate insulin permeability. They prepared and nicotinamide-immobilized gel membranes separately. (VIPUL P. PATEL n.d.)

2) Inflammation-induced pulsatile release: When human beings receive physical or chemical stress, such as injury, broken bones, etc., inflammation reactions take place at the injured sites. At the inflammatory sites, inflammation-responsive phagocytic cells, such as macrophages and polymorphonuclear cells, play a role in the healing process of the injury. During inflammation, hydroxyl radicals (OH) are produced from these inflammation-responsive cells. Yui and co-workers. used hyaluronic acid (HA), a linear mucopolysaccharide composed of repeating disaccharide subunits of N-acetyl-D-glucosamine and D-guluronic acid. In the body, HA is mainly degraded either by a specific enzyme, hyaluronidase, or hydroxyl radicals. Degradation of HA via the hyaluronidase is very low in a normal state of health. Degradation via hydroxyl radicals however, is usually dominant and rapid when HA is injected at inflammatory sites. Thus,Yui and co-workers prepared cross-linked HA with ethyleneglycol diglycidylether or polyglycerol polyglycidylether. These HA gels degraded only when the hydroxyl radicals were generated through the Fenton reaction between Fe+2 ions and hydrogen peroxide in vitro. Thus, a surface erosion type of degradation was achieved. When microspheres were incorporated in the HA hydrogels as a model drug, these microspheres were released only when hydroxyl radicals induced HA gel degradation. The microsphere release was regulated by the surface erosion type of degradation. (Mayee RV 2012)

3) Drug release from intelligent gels responding to antibody concentration:

There are numerous kinds of bioactive compounds which exist in the body. Recently, novel gels were developed which responded to the change in conbecentration of bioactive compounds to alter their swelling/deswelling characteristics. Miyata and co-workers focused on the introduction of stimuli-responsive cross-linking structures into hydrogels. Special attention was given to antigen antibody complex formation as the cross-linking units in the gel, because specific antigen recognition of an antibody can provide the basis for a new device fabrication. (VIPUL P. PATEL1 n.d.)

4) pH sensitive drug delivery system:

pH- sensitive polymers are polyelectrolytes that bear in their structure weak acidic or basic groups that either accept or release protons in response to changes in environmental pH. Examples of pH dependent polymers include cellulose acetate phthalate, polyacrylates, sodium carboxy methyl cellulose. (GANESH RASVE 2011)

III. Externally regulated pulsatile release system:

This system is not self-operated, but instead requires externally generated environmental changes to initiate drug delivery. These can include magnetic fields, ultrasound, electric field, light, and mechanical force. (GANESH RASVE 2011)

A. Magnetic induces release:

Magnetic carriers receive their magnetic response to a magnetic field from incorporated materials such as magnetite, iron, nickel, cobalt etc. Magnetic-sensitive behavior of intelligent ferrogels for controlled release of drug was studied by Tingyu Liu, et al. An intelligent magnetic hydrogel (ferrogel) was fabricated by mixing poly (vinyl alcohol) (PVA) hydrogels and Fe_3O_4 magnetic particles through freezing-thawing Cycles. Although the external direct current magnetic field was applied to the ferrogel, the drug got accumulated around the ferrogel, but the accumulated drug spurt to the environment instantly when the magnetic fields instantly switched “off”. Furthermore, rapid slow drug release can be tunable while the magnetic field was switched from “off” to “on” mode. The drug release behavior from the ferrogel is strongly dominated by the particle size of Fe_3O_4 under a given magnetic field36. Tingyu Liu, et al developed the magnetic hydrogels which was successfully fabricated by chemically crosslinking of gelatin hydrogels and Fe_3O_4 nanoparticles (ca.

40–60 nm) through genipin (GP) as cross-linking agent. (GANESH RASVE 2011)

B. Ultrasound induces release:

Ultrasound is mostly used as an enhancer for the improvement of drug permeation through biological barriers, such as skin. The interactions of ultrasound withbiological tissues are divided into two broad categories: thermal and nonthermal effects. Thermal effects are associated with the absorption of acoustic energy by the fluids or tissues38. Non-thermal bio-effects are generally associated with oscillating or cavitating bubbles, but also include noncavitation effects such as radiation pressure, radiation torque, and acoustic streaming. (GANESH RASVE 2011)

C. Electric field induces release:

Electrically responsive delivery systems are prepared by polyelectrolytes (polymers which contain relatively high concentration of ionisable groups along the backbone chain) and are thus, pH-responsive as well as electro-responsive. Under the influence of electric field, electro-responsive hydrogels generally bend, depending on the shape of the gel which lies parallel to the electrodes whereas deswelling occurs when the hydrogel lies perpendicular to the electrodes. An electroresponsive drug delivery system was developed by R. V. Kulkarni, et al., using poly(acrylamide-grafted-xanthan gum) (PAAm-g-XG) hydrogel for transdermal delivery of ketoprofen. (GANESH RASVE 2011)

D. Light induces release:

Light-sensitive hydrogels have potential applications in developing optical switches, display units, and opthalmic drug delivery devices. The interaction between light and material can be used to modul drug delivery. When hydrogel absorb the light and convert it to heat, raising the temperature of composite hydrogel above its LCST41, hydrogel collapses and result in an increased rate of release of soluble drug held within the matrix. (GANESH RASVE 2011)

IV. Multipaticulate system:

Recent trends indicate that multiparticulate drug delivery systems are especially suitable for achieving controlled or delayed release oral formulations with low risk of dose dumping, flexibility of blending to attain different release patterns as well as reproducible and short gastric residence time. Such systems are reservoir type with either

rupturable or altered permeability coating and generally housed in capsular body.

The purpose of designing multiparticulate dosage form is to develop a reliable formulation that has all the advantages of a single unit formulation and yet devoid of the danger of alteration in drug release profile and formulation behaviour due to unit to unit variation.

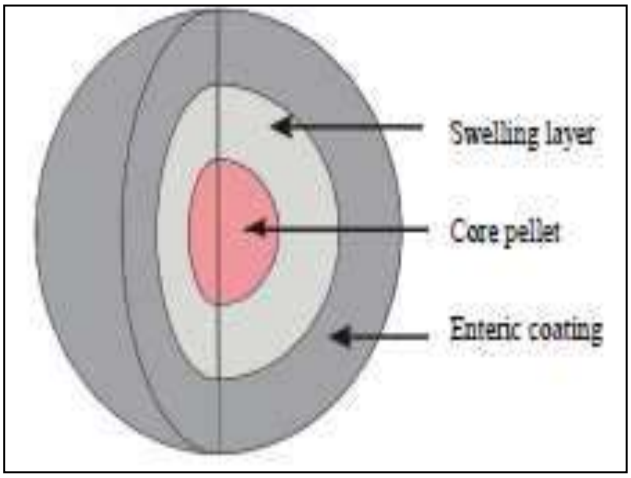


Fig. 14: Hypothetical design of a multiparticulate pulsatile system. (GANESH RASVE 2011)

Andrei Dashevsky, et al. developed a pulsatile multiparticulate drug delivery system (DDS), coated with aqueous dispersion of Aquacoat® ECD. A rupturable pulsatile drug delivery system consists of (i) a drug core; (ii) a swelling layer, comprising a superdisintegrant and a binder; and (iii) an insoluble, water-permeable polymeric coating43. Upon water ingress, the swellable layer expands, resulting in the rupturing of outer membrane with subsequent rapid drug release. Regarding the cores, the lag time was shorter; theophylline was layered onsugar cores compared with cores consisting of theophylline. Regarding swelling layer, the release after lag time was fast and complete. Drug release was achieved after the lag time, when low-substituted hydroxypropyl cellulose (L-HPC) and sodium starch glycolate (Explotab®) were used as swelling agents. Outer membrane, formed using aqueous dispersion Aquacoat® ECD was brittle and ruptured sufficiently to ensure fast drug release, compared to ethylcellulose membrane formed using organic solution. The addition of talc led to increase brittleness of membrane and was very advantageous.

Drug release starts only after rupturing of outer membrane. C. Sun, et al. developed novel pHsensitive copolymer microspheres containing methylacrylic acid and styrene cross-linking with divinylbenzene were synthesized by free radical polymerization. The copolymer microspheres showed pulsatile swelling behavior when the pH of the media changed. The pHsensitive microspheres were loaded with diltiazem hydrochloride (DH). The release characteristics of the free drug and the drug-loaded microspheres were studied under both simulated gastric conditions and intestinal pH conditions. The in vivo evaluation of the pulsatile preparation was subsequently carried out using beagle dogs. Sigmoidal Release System: This consists of pellet cores containing drug and succinic acid coated with ammoniomethacrylate copolymer USP/NF type B. The lag time is controlled by the rate of water influx through the polymer membrane. The water dissolves succinic acid, and the drug in the core and the acid solution in turn increases permeability of the hydrated polymer film. In addition to succinic acid, acetic acid, glutaric acid, tartaric acid, malic acid, or citric acid can be used. The increased permeability can be explained by improved hydration of film, which increases free volume. These findings were used to design a coated delivery system with an acidcontaining core. The in-vitro lag time correlated well with in-vivo data when tested in beagle dogs. (GANESH RASVE 2011)

5. Evaluation:

In vitro release profile:

In vitro dissolution profile of each formulation was determined by employing USP XXIII apparatus by rotating basket method in different media like stimulated gastric fluid pH 1.2 buffer for 2 hours (since the average gastric emptying time is 2hrs), stimulated intestinal fluid pH 7.4 buffer for 3hours (average small intestinal transit time is 3hrs) and colonic fluid pH 6.8 buffer for subsequent hours. The dissolution media were maintained at a temperature of 37± 1°C, the speed rotation of basket maintained were 50rpm. Microsphere equivalent to 40 mg TMH modified pulsing capsules were placed in basket in each dissolution vessel to prevent floating.5ml of the samples withdrawn from dissolution media at suitable intervals and same amount was replaced with fresh buffer. The absorbance was measured at 269 nm. (A. BHAT 2011)

Determination of drug content in pellets:

In a 100 ml volumetric flask, 25 mg of crushed microcapsules were taken, and volume was made up to mark with pH 6.8. The flask was shaken for 12 hrs using an orbital shaker incubator. Then the solution was filtered and from the filtrate appropriate dilutions were made and absorbance was measured at 272 nm by using UV absorption spectroscopy. (A. BHAT 2011)

Determination of particle size and external morphology:

Determination of average particle size of theophylline pellets was carried out by optical microscopy Scanning Electron Microphotography (SEM) studies was carried out by using JEOL JSM T-330 'A' Scanning microscope (Japan). Dry pellets were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microcapsules were taken by random scanning of the stub. (A. BHAT 2011)

Determination of the time of erosion of the plugs:

The time for complete erosion of the plugs (compressed plugs) was determined with a disintegration testing apparatus (Cintex Industrial Corporation, Dadar, Mumbai), (900 ml pH 6.8 phosphate buffer USP XXIII, 37±0.50C). (A. BHAT 2011)

Determination of swelling index of plugs:

The plugs prepared with varying compression pressures were tested for swelling index using disintegration apparatus (900 ml pH 7.4 phosphate buffer USP XXIII, 37±0.5 □C). Xanthan gum polymer was taken as model polymer in order to determine swelling rate. (A. BHAT 2011)

Evaluation of thickness of designed pulsatile capsule:

The thickness of the Ethyl cellulose coating was measured using screw gauge and was expressed in mm. 10 capsules were selected randomly from each batch and weighed individually for weight variation. The test requirements are met if none of the individual weights are less than 90% or more than 110% of the average. (A. BHAT 2011)

Stability studies:

Stability studies as per ICH guidelines were carried out for optimized formulation from each polymer batch were selected. This includes storage at both normal and exaggerated temperature conditions, with the necessary extrapolations to ensure the product will, over its designed shelf life, provide medication for absorption at the same rate as when originally formulated. Stability samples are stored at accelerated stability studies: 40±2°C/75±5% RH, intermediate stability studies: 30±2°C/65±5% RH, long term stability studies: 25±2°C/60±5% RH. The selected formulations were stored at above mentioned conditions for a period of three months. At weekly intervals, formulations were observed for any physical changes during the period of storage. At the end of third month the formulations were analysed for the analysed for the drug content and dissolution profiles. (A. BHAT 2011)

7. Recent Advances In Chronopharmaceutical DDS:

CONTIN technology:

In this technology, molecular coordination complexes are formed between a cellulose polymer and a non-polar solid aliphatic alcohol optionally substituted with an aliphatic group by solvating the polymer and reacting the cellulose polymer directly with an aliphatic alcohol. This technology has concretely enabled the tablet forms of sustained release aminophylline, theophylline, morphine. This technology provides for closer control over the amount drug released to the bloodstream and benefits patients in terms of reducing the number of doses they need to take every day, providing more effective control of their disease and reducing unwanted side effects. (PRITI P. PEDNEKAR 2011)

OROS Technology:

Chronoset™ is proprietary OROS® (Osmotic-controlled Release Oral delivery System) developed by Alza Corporation (now part of Johnson and Johnson). The system is composed of two compartments—the drug vessel and the osmotic engine cap. When the system is exposed to an aqueous medium, water permeates into the osmotic engine cap via a ratecontrolling membrane. Hydration of the osmotic engine leads to its expansion, which exerts a driving force against the ridge of the drug vessel. The two compartments separate from each other by sliding apart. After disengaging, the open mouth of the drug vessel is exposed to the fluid environment. The Chronoset® can deliver essentially the entire dose and minimizes the drug residue in the drug vessel after the operation. The vessel is made of waterimpermeable ethylene-co-vinyl acetate copolymer (EVA), while the cap is made of proprietary water-permeable blends of polycaprolactone (TONE) and flux enhancers. (Maroni A 2005)

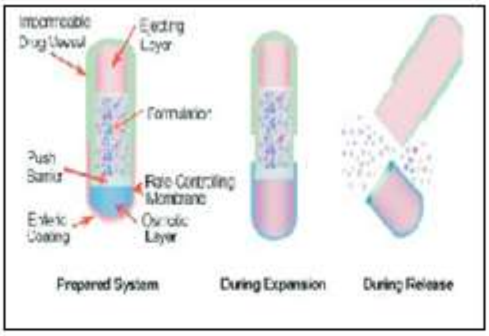


Fig.15: OROS Technology (PRITI P. PEDNEKAR 2011)

CEFORM® technology:

Ceform® allows the production of uniformly sized and shaped microspheres of pharmaceutical compounds. These microspheres are almost perfectly spherical, having a diameter that is typically 150 to 180 mm, and allow for high drug content. The microspheres can be used in a wide variety of dosage forms, including tablets, capsules, suspensions, effervescent tablets, and sachets. The microspheres can be coated for controlled release (Ceform CR), provided with an enteric coating (Ceform EC), or combined into a fast/slow release combination (Ceform EA/CR) (PRITI P. PEDNEKAR 2011).

DIFFUCAPS® technology:

Diffucaps is a multiparticulate bead system comprised of multiple layers of drug, excipients, and release-controlling polymers. The beads contain a layer of organic acid or alkaline buffer to control the solubility of a drug by creating an optimal pH microenvironment for drugs that exhibit poor solubility in intestinal pH, in environments with pH greater than 8.0, or in physiological fluids. Alternatively, the beads can contain a solid-solution of drug and crystallization inhibitor to enhance bioavailability by maintaining the drug in its amorphous state. Each Diffucaps bead has an inert core surrounded by drug and coated with a functional polymer membrane to control the rate of drug release. Diffucaps beads are <1.5 mm in diameter and can be filled into capsules or compressed into orally disintegrating tablets. (PRITI P. PEDNEKAR 2011)

Advantages of Diffucaps:

- Ideal for drugs exhibiting poor solubility in lower intestinal pH, in environments with pH above 8.0, or in physiological fluids
- Can combine multiple drugs and/or multiple release profiles in the same dosage form
- Simple formulation of dose-proportional strengths.
- Can minimize food effect. (PRITI P. PEDNEKAR 2011)

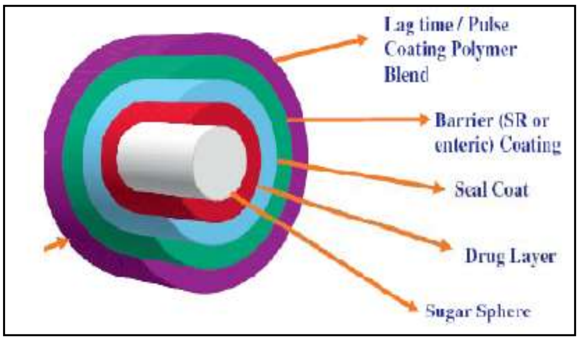

Lag time / Pulse Coating Polymer Blend
Barrier (SR or enteric) Coating
Seal Coat
Drug Layer
Sugar Sphere

Fig.16: Cross section of Diffucaps (PRITI P. PEDNEKAR 2011)

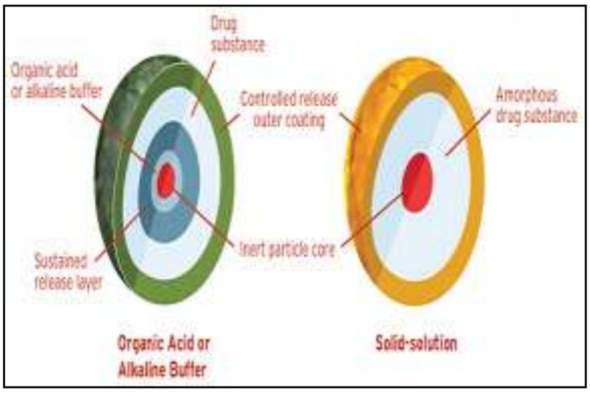

Drug substance
Organic acid or alkaline buffer
Controlled release outer coating
Amorphous drug substance
Inert particle core
Sustained release layer
Organic Acid or Alkaline Buffer
Solid-solution

Fig.17: Diffucap

CHRONOTOPIC® technology:

It consists of a core containing drug reservoir coated by a hydrophilic polymer hydroxylpropylmethylcellulose (HPMC). An additional enteric-coated film is given outside this layer to overcome intra-subject variability in gastric emptying rates. The lag time and the onset of action are controlled by the thickness and the viscosity grade of HPMC. (PRITI P. PEDNEKAR 2011)

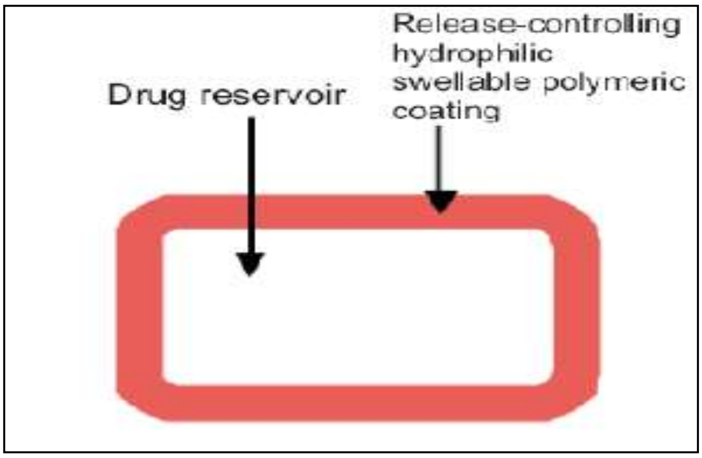


Fig.18: Chronotopic Technology (PRITI P. PEDNEKAR 2011)

EGALET® technology (Egalet Ltd, Denmark):

It offers a delayed release. System consists of an impermeable shell with two lag plugs, active drug is sandwiched between the plugs. After the inert plugs have eroded, the drug is released, thus a lagtime occurs. Time of release can be modulated by the length and composition of the plugs. The shells are made of slowly biodegradable polymers (such as ethylcellulose) and include plasticizers (such as cetostearyl alcohol), while the matrix of the plugs is made up of a mixture of pharmaceutical excipients including polymers like polyethylene oxide (PEO). (PRITI P. PEDNEKAR 2011)

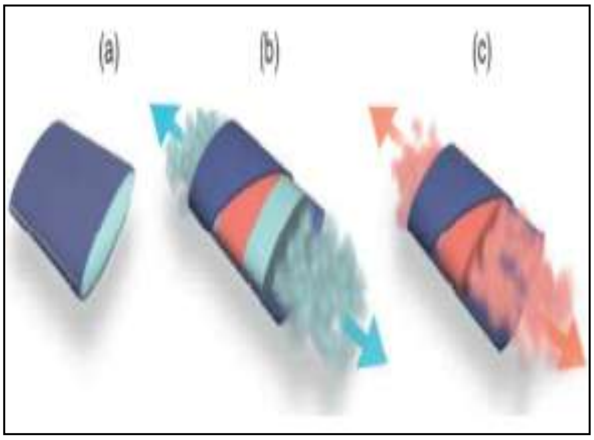


Fig.19: Egalet: (a) Intact Unit (b) Erosion of the time delay insert plugs, and (c) Drug release (PRITI P. PEDNEKAR 2011)

CODAS® technology (Elan Drug Technologies, USA):

CODAS(Chronotherapeutic Oral Drug Absorption System) are a multiparticle system designed for bedtime drug dosing, incorporating a 4–5-hour delay in drug delivery. This delay is introduced by the level of nonenteric release-controlling polymer applied to drug-loaded beads. The rate of release is essentially independent of pH, posture, and food. GeoClock® technology (Skye Pharma PLC, UK) Geoclock® tablets have an active drug inside an outer tablet layer consisting of a mixture of hydrophobic wax and brittle material in order to obtain a pH-independent lag time prior to core drug delivery at a predetermined release rate. (PRITI P. PEDNEKAR 2011)

GeoClock® technology:

The concept is designed on the basis of Geomatrix technology. Initially a multilayer technology was recommended for constant drug release in this technology. The active core or hydrophilic matrix is coated partially on one or both bases. This partial coating adjusts the core hydration process and minimizes the surface area available for drug release. In presence of dissolution medium the barrier layer swells and becomes gel. This gelling layer is not eroded but acts as a modulating membrane to control release process. The erodible surface is instead progressively removed by the dissolution medium.

Upon erosion more planar surface(s) of the active core is exposed with increasing time to outer environment which helps drug release. (A. S. Mandal 2010)

TIMERx® technology:

It is hydrogel based controlled release device. This technology can provide from zero order to chronotherapeutic release. It can provide different release kinetic by manipulating molecular interactions. The authors claimed that the “molecular engine” replaces the need for complex processing or novel excipients and allows desired drug release profiles to be “factory set” following a simple formulation development process. Basically, this technology combines primarily xanthan and locust bean gums mixed with dextrose. The physical interaction between these components works to form a strong, binding gel in the presence of water. Drug release is controlled by the rate of water penetration from the gastrointestinal tract into the TIMERx gum matrix, which expands to form a gel and subsequently releases the active drug substance. (A. S. Mandal 2010)

8.Marketed Technologies Of Chronopharmaceutical DDS:

Technology	Mechanism	Proprietary name and dosage form	API	Disease
OROS*	Osmotic mechanism	Covera-H5*; XL tablet	Verapamil HCL	Hypertension
Three dimentional printing	Externally regulated system	Their Form*	Diclofenac sodium	Inflammation
DIFFUCAPS*	Multiparticulate system	Innopran*; XL Tablets	Verapamil HCL, Propranoll	Hypertension
Pulsincap TM	Rupturable system	PulsincapTM	Dofetilide	Hypertension
Physico-chemical modification of API	-	Zocor® tablets	Simvastatin	Hypercholesterolemia

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SELF DISPERSING FORMULATIONS (SDFs)

Deshpande Tanvee, Mogal Rajendra

INTRODUCTION:

Recently synthesized drug that are being discovered are lipophillic in nature and have poor aqueous solubility, thereby posing problems in their formulation into delivery systems. Because of their low aqueous solubility and low permeability, dissolution and/or release rate from the delivery system forms the rate-limiting step in their absorption and systemic availability. More than 60% of potential drug products suffer from poor water solubility. For the therapeutic delivery of lipophillic active moieties (BCS class II drugs), lipid based formulations are inviting increasing attention. Currently a number of technologies are available to deal with the poor solubility, dissolution rate and bioavailability of insoluble drugs.

The Self-Dispersing Formulations (SDFs) is one of the promising approaches to overcome the formulation difficulties of various hydrophobic/ lipophillic drugs and to improve the oral bioavailability of poorly absorbed drugs. The SDFs contain oil and a surfactant mixture into which the drug is incorporated. They emulsify when mixed with aqueous environment. The self emulsification process is specific to the particular pair of oil and surfactant, surfactant concentration, oil/surfactant ratio, and the temperature at which self-emulsification occurs. After self dispersion, the drug is rapidly distributed throughout the gastrointestinal tract as fine droplets. Bioavailability enhancement results from the finely dispersed state of the drug containing lipid globules. The large surface area enhances the dissolution. The emulsion globules are further solubilized in the gastrointestinal tract by bile fluids. The presence of surfactant causes enhanced absorption due to membrane induced permeation changes. The droplets formed are either positively charged or negatively charged. As the mucosal lining is negatively charged it was observed that positively charged particles penetrated deeper into the ileum. A cationic emulsion has greater bioavailability than an anionic emulsion. The SDFs are of two kinds namely, Self-Emulsifying Drug Delivery Systems (SEDDS) formed using surfactants of HLB < 12 and Self-Micro Emulsifying Drug Delivery Systems (SMEDDS) formed with surfactants of HLB > 12. Both SEDDS and SMEDDS are stable preparations and improve the dissolution of the drug due to increased surface area on dispersion. Therefore, they are not dependent on bile secretion for absorption. The emulsified form itself is readily absorbable. This ensures a rapid transport of poorly soluble drugs into the blood.

The SDF system is a liquid-based formulation rather than a semisolid formulation and, therefore, an alternative approach are required. The concept works by the adsorption/absorption of a liquid SDF onto a neutral carrier (i.e., neutral silicate). Although surprisingly straightforward, developing this solid dosage form technique has required extensive investigation of critical success parameters including:

1. Extensive screening of different neutral carriers to evaluate their ability to adsorb maximum levels of the liquid SELF.

2. Maximum loading value of the carrier and effect on tablet compression.

3. Absorption onto the carrier and effect on flowability — an essential feature for tablet compression.

4. Evaluation of the integrity of the system with a poorly soluble API to examine the effect of transforming a liquid into a powder on drug solubility and dissolution rate.

Self emulsifying drug delivery systems (SEDDS) and self micro emulsifying drug delivery systems (SMEDDS) are defined as isotropic mixtures of natural synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents/surfactants that have a unique ability of forming fine oil-in-water (o/w) micro emulsions upon mild agitation followed by dilution in aqueous media, such as GI fluids. the basic difference between SEDDS and SMEDDS is that SEDDS typically produce opaque emulsions with a droplet size between 100 and 300 nm while SMEDDS form transparent micro emulsions with a droplet size of less than 50 nm also the concentration of oil in SMEDDS is less than 20% as compared to 40-80% in SEDDS. When compared to emulsions, which are sensitive and metastable dispersed forms, SMEDDS are physically stable formulations that are easy to manufacture. (Kyatanwar 2010) Thus, for lipophillic drug compounds that exhibit dissolution rate limited absorption, these systems may offer an improvement in the rate and extent of absorption and result in more reproducible blood-time profile.

In the present topic focus will be on lipophillic drug based delivery systems. Emulsion particles can be of either micro or nano size depending on the composition of the system. These formulations circumvent the dissolution step in the gastro-intestinal tract, but are still dependent on digestion. (S 2011)

NEED OF SDFs:

Oral delivery of poorly water soluble compounds is to pre dissolve the compound in a suitable solvent and fills the formulation into capsules. (Patel 2008)The main benefit of this approach is that pre dissolving the compound overcomes the initial rate limiting step of particulate dissolution in the aqueous environment within the GI tract. However, a potential problem is that the drug may precipitate out of solution when the formulation disperses in the GI tract, Particularly if a hydrophilic solvent is used (eg. polyethylene glycol).if the drug can be dissolved in a lipid vehicle there is less potential for precipitation on dilution in the GI tract, as partitioning kinetics will favour the drug remaining in the lipid droplets.

Another strategy for poorly soluble drugs is to formulate in a solid solution using a water soluble polymer to aid solubility of the drug compound. For example polyvinyl pyrrolidone (PVP) and polyethylene glycol (PEG 6000) have been used for preparing solid solutions with poorly soluble drugs. One potential problem with this type of formulation is that the drug may favour\r a more thermodynamically stable state, which can result in the compound crystallizing in the polymer matrix. Therefore the physical stability of such formulations needs to be assessd using techniques such as differential scanning calorimetry or X-ray crystallography. In this type of case SEDD system is a good option.

ADVANTAGES OF SDFs:

- *Improvement in oral bioavailability:*

Dissolution rate dependant absorption is a major factor that limits the bioavailability of numerous poorly water soluble drugs. (Shukla 2010)The ability of SMEDDS to present the drug to GIT in solubilised and micro emulsified form (globule size between 1-100 nm) and subsequent increase in specific surface area enable more efficient drug transport through the intestinal aqueous boundary layer and through the absorptive brush border membrane leading to improved bioavailability. E.g. In case of halofantrine approximately 6-8 fold increase in bioavailability of drug was reported in comparison to tablet formulation. (Tang 2007)

- *Ease of manufacture and scale-up:*

Ease of manufacture and scale- up is one of the most important advantages that make SMEDDS unique when compared to other drug delivery systems like solid dispersions, liposomes, nanoparticles, etc., dealing with improvement of bio-availability. SMEDDS require very simple and economical manufacturing facilities like simple mixer with agitator and volumetric liquid filling equipment for large-scale manufacturing. This explains the interest of industry in the SMEDDS.

- *Reduction in inter-subject and intra-subject variability and food effects:*

There are several drugs which show large inter-subject and intra-subject variation in absorption leading to decreased performance of drug and patient non-compliance. Food is a major factor affecting the therapeutic performance of the drug in the body. (Khoo 1998) SMEDDS are a boon for such drugs. Several research papers specifying that, the performance of SMEDDS is independent of food and, SMEDDS offer reproducibility of plasma profile are available.

- *Ability to deliver peptides that are prone to enzymatic hydrolysis in GIT:*

One unique property that makes SMEDDS superior as compared to the other drug delivery systems is their ability to deliver macromolecules like peptides, hormones, enzyme substrates and inhibitors and their ability to offer protection from enzymatic hydrolysis. The intestinal hydrolysis of prodrug by cholinesterase can be protected if Polysorbate 20 is emulsifier in micro emulsion formulation. These systems are formed spontaneously without aid of energy or heating thus suitable for thermo labile drugs such as peptides (Cortesi 1997).

- *No influence of lipid digestion process:*

Unlike the other lipid-based drug delivery systems, the performance of SMEDDS is not influenced by the lipolysis, emulsification by the bile salts, action of pancreatic lipases and mixed micelle formation. SMEDDS are not necessarily digested before the drug is absorbed as they present the drug in micro-emulsified form which can easily penetrate the mucin and water unstirred layer.

- *Increased drug loading capacity:*

SMEDDS also provide the advantage of increased drug loading capacity when compared with conventional lipid solution as the solubility of poorly water soluble drugs with intermediate partition coefficient ($2<\log P>4$) are typically low in natural lipids and much greater in ampiphihillic surfactants, co surfactants and co-solvents.

ADVANTAGES OF SDFs OVER EMULSION:

- SMEDDS not only offer the same advantages of emulsions of facilitating the solubility of hydrophobic drugs, but also overcomes the drawback of the layering of emulsions after sitting for a long time. SMEDDS can be easily stored since it belongs to a thermodynamics stable system. (Shukla 2010)

- Microemulsions formed by the SMEDDS exhibit good thermodynamics stability and optical transparency. The major difference between the above microemulsions and common emulsions lies in the particle size of droplets. The size of the droplets of common emulsion ranges between 0.2 and 10 μm, and that of the droplets of microemulsion formed by the SMEDDS generally ranges between 2 and 100 nm (such droplets are called droplets of nano particles).Since the particle size is small, the total surface area for absorption and dispersion is significantly larger than that of solid dosage form and it can easily penetrate the gastrointestinal tract and be absorbed. The bioavailability of the drug is therefore improved.

- SMEDDS offer numerous delivery options like filled hard gelatin capsules or soft gelatin capsules or can be formulated in to tablets whereas emulsions can only be given as an oral solutions. (Kawakami 2002)

- Emulsion cannot be autoclaved as they have phase inversion temperature, while SMEDDS can be autoclaved.

DRAWBACKS OF SDFs:

- One of the obstacles for the development of SMEDDS and other lipid-based formulations is the lack of good predicative *in vitro* models for assessment of the formulations. (Wakerly 1986)

- Traditional dissolution methods do not work, because these formulations potentially are dependent on digestion prior to release of the drug.

- This *in vitro* model needs further development and validation before its strength can be evaluated.

- Further development will be based on *in vitro - in vivo* correlations and therefore different prototype lipid based formulations needs to be developed and tested *in vivo* in a suitable animal model.

- The drawbacks of this system include chemical instabilities of drugs and high surfactant concentrations in formulations (approximately 30-60%) which irritate GIT.
- Moreover, volatile co solvents in the conventional self-micro emulsifying formulations are known to migrate into the shells of soft or hard gelatin capsules, resulting in the precipitation of the lipophillic drugs (Murdandea 2008).
- The precipitation tendency of the drug on dilution may be higher due to the dilution effect of the hydrophilic solvent Formulations containing several components become more challenging to validate.

FORMULATION OF SELF DISPERSING SYSTEMS:

A. COMPOSITION OF SDFs:

Pouton revealed that the self-emulsification process depends on:

- The nature of the oil–surfactant pair
- The surfactant concentration and oil/surfactant ratio
- The temperature at which self-emulsification occurs.
- The types of oils used.

1. *OILS*:

The oil represents one of the most important excipients in the SMEDDS formulation not only because it can solubilize the required dose of the lipophillic drug or facilitate self emulsification but also and mainly because it can increase the fraction of lipophillic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract depending on the molecular nature of the triglyceride (Kimura 1994). Both long and medium chain triglyceride (LCT and MCT) oils with different degrees of saturation have been used for the design of self-emulsifying formulations. Furthermore, edible oils which could represent the logical and preferred lipid excipient choice for the development of SMEDDS are not frequently selected due to their poor ability to dissolve large amounts of lipophillic drugs. Modified or hydrolyzed vegetable oils have been widely used since these excipients form good emulsification systems with a large number of surfactants approved for oral administration and exhibit better drug solubility properties (Murdandea 2008). They offer formulative and physiological advantages and their degradation products resemble the natural end products of intestinal digestion. Novel semisynthetic medium chain derivatives, which can be defined as ampiphillic compounds with surfactant properties, are progressively and effectively replacing the regular medium chain triglyceride oils in the SMEDDS. (Lawrence 2000) This is in accordance with findings of Deckelbaum (1990) showing that MCT is more soluble and have a higher mobility in the lipid/water interfaces than LCT associated with a more rapid hydrolysis of MCT. In general, when using LCT, a higher concentration of cremophor RH40 was required to form microemulsions compared with MCT.

2. *SURFACTANTS:*

Several compounds exhibiting surfactant properties may be employed for the design of self-emulsifying systems, but the choice is limited as very few surfactants are orally acceptable. The most widely recommended ones being the non-ionic surfactants with a relatively high hydrophillic-lipophillic balance (HLB). The commonly used emulsifiers are various solid or liquid ethoxylated polyglycolyzed glycerides and polyoxyethylene 20 oleate. Safety is a major determining factor in choosing a surfactant. Emulsifiers of natural origin are preferred since they are considered to be safer than the synthetic surfactants. However, these surfactants have a limited self-emulsification capacity. Non-ionic surfactants are less toxic than ionic surfactants but they may lead to reversible changes in the permeability of the intestinal lumen. The lipid mixtures with higher surfactant and co-surfactant/oil ratios lead to the formation of SMEDDS. There is a relationship between the droplet size and the concentration of the surfactant being used. In some cases, increasing the surfactant concentration could lead to droplets with smaller mean droplet size, this could be explained by the stabilization of the oil droplets as a result of the localization of the surfactant molecules at the oil-water interface. On the other hand, in some cases the mean droplet size may increase with increasing surfactant concentrations (Hauss 1998). This phenomenon could be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the increased surfactant concentration and leading to ejection of oil droplets into the aqueous phase. The surfactants used in these formulations are known to improve the bioavailability by various mechanisms including: improved drug dissolution, increased intestinal epithelial permeability, increased tight junction permeability and decreased/inhibited p-glycoprotein drug efflux. However, the large quantity of surfactant may cause moderate reversible changes in intestinal wall permeability or may irritate the GI tract. Formulation effect and surfactant concentration on gastrointestinal mucosa should ideally be investigated in each case. Surfactant molecules may be classified based on the nature of the hydrophilic group within the molecule. The four main groups of surfactants are defined as follows,

a) *Anionic Surfactants*: where the hydrophilic group carries a negative charge such as carboxyl (RCOO-), sulphonate (RSO3-) or sulphate (ROSO3-). Examples: Potassium laurate, sodium lauryl sulphate. (Karim 1994)

b) *Cationic surfactants*: where the hydrophilic group carries a positive charge. Example: quaternary ammonium halide.

c) *Ampholytic surfactants* (also called zwitterionic surfactants): contain both a negative and a positive charge. Example: sulfobetaines.

d) *Nonionic surfactants*: where the hydrophilic group carries no charge but derives its water solubility from highly polar groups such as hydroxyl or polyoxyethylene (OCH2CH2O). Examples: Sorbitan esters (Spans), polysorbates (Tweens).

3. *CO-SOLVENTS:*

The production of an optimum SMEDDS requires relatively high concentrations (generally more than 30% w/w) of surfactants, thus the concentration of surfactant can be reduced by incorporation of co surfactant. Role of the co-surfactant together with the surfactant is to lower the interfacial tension to a very small even transient negative value. At this value the interface would expand to form fine dispersed droplets, and subsequently adsorb more surfactant and surfactant/co-surfactant until their bulk condition is depleted enough to make interfacial tension positive again. This process known as ‘spontaneous emulsification’ forms the microemulsion. However, the use of co-surfactant in self emulsifying systems is not mandatory for many non-ionic surfactants. The selection of surfactant and co-surfactant is crucial not only to the formation of SMEDDS, but also to solubilization of the drug in the SMEDDS. Organic solvents, suitable for oral administration (ethanol, propylene glycol (PG), polyethyleneglycol (PEG), etc) may help to dissolve large amounts of either the hydrophilic surfactant or the drug in the lipid base and can act as co-surfactant in the self emulsifying drug delivery systems, although alcohol- free self-emulsifying microemulsions have also been described in the literature. Indeed, such systems may exhibit some advantages over the previous formulations when incorporated in capsule dosage forms, since alcohol and other volatile co-solvents in the conventional self-emulsifying formulations are known to migrate into the shells of soft gelatin or hard sealed gelatin capsules resulting in the precipitation of the lipophilic drug. On the other hand, the lipophilic drug dissolution ability of the alcohol free formulation may be limited. Hence, proper choice has to be made during selection of components.

4. *CO-SURFACTANTS*:

In SMEDDS, generally co-surfactant of HLB value 10-14 is used. Hydrophilic co-surfactants are preferably alcohols of intermediate chain length such as hexanol, pentanol and octanol which are known to reduce the oil water interface and allow the spontaneous formulation of micro emulsion.

5. CONSISTENCY BUILDER:

Additional material can be added to alter the consistency of the emulsions; such materials include tragacanth, cetyl alcohol, stearic acids and /or beeswax, etc.

6. POLYMERS:

Inert polymer matrix representing from 5 to 40% of composition relative to the weight, which is not ionizable at physiological pH and being capable of forming matrix are used. Examples are hydroxyl propyl methyl cellulose, ethyl cellulose, etc.

B. THE EMULSIFICATION PROCESS:

Self-emulsification is a phenomenon which has been widely exploited commercially in formulations of emulsifiable concentrates of herbicides and pesticides. Concentrates of crop-sprays are to be diluted by the user, such as farmers or house-hold gardeners, allowing very hydrophobic compounds to be transported efficiently. In contrast, SMEDDS, using excipients acceptable for oral administration to humans, have not been widely exploited and knowledge about their physicochemical principles is therefore limited.

(a) Mechanism of Self Emulsification:

In emulsification process the free energy (ΔG) associated is given by the equation:

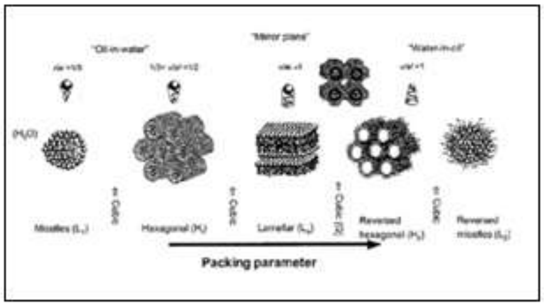
$\Delta G = \Sigma Ni\pi ri$ ------------------------------ (1)

In which 'N' is Number of droplets with radius 'r' and 'σ' is interfacial energy. It is apparent from equation that the spontaneous formation of the interface between the oil and water phases is energetically not favored. The system commonly classified as SEDDS have not yet been shown to emulsify spontaneously in the thermodynamic sense. The process of self-emulsification was observed using light microscopy. Groves and Mustafa developed method of quantitatively assessing the ease of emulsification by monitoring the turbidity of the oil-surfactant in a water stream using phosphated nonylphenoloxylate (PNE) and phosphated fatty alcohol ethoxlate (PFE) in nhexane (Swenson 1994).

Pouton has argued that the emulsification properties of the surfactant may be related to phase inversion behavior of the system. For example, on increase the temperature of an oil in water system stabilized using nonionic surfactant, the cloud point of the surfactant will be reached followed by phase inversion. The surfactant is highly mobile at the phase inversion temperature; hence the o/w interfacial energy is minimized leading to a reduction in energy required to cause emulsification. The specificity of surfactant combination required to allow spontaneous emulsification may be associated with a minimization of the phase inversion temperature, thereby increasing the ease of emulsion. Phase studies are also necessary for liquid crystal formation in selfemulsification. These indicate that good formulations are usually operating close to a phase inversion region and in a region of enhanced close to a phase inversion region and in a region of enhanced aqueous solubilization. In the phase diagram of the system (30 % w/w tween and 85/70 % w/w MCT oil) for dilution in water over a range of temperature shows that the phase inversion region is at approximately 40° C and the system works well at ambient temperature up to 60°C above which water in oil emulsion tend to form. The emulsification process may be associated with the ease with which water penetrates the oil-water interface with the formation of liquid crystalline phases resulting in swelling at the interface thereby resulting in greater ease of emulsification. However, for system containing co- surfactant, significant partitioning of components between the oil and aqueous phases may take place leading to a mechanism described as "diffusion and stranding", where by the oil is solubilized, leading to migration in to the aqueous phase.

(b) Dilution phases:

Upon dilution of a SMEDDS formulation, the spontaneous curvature of the surfactant layer changes via a number of possible liquid crystalline phases. The droplet structure can pass from a reversed spherical droplet to a reversed rod-shaped droplet, hexagonal phase, lamellar phase, cubic phase and various other structures until, after appropriate dilution, a spherical droplet will be formed again . Representation of the of commonly encountered phases upon addition of water to an oil-surfactant combination. (Gursoy 2004)



BIOPHARMACEUTICAL ASPECTS OF SDFs:

The lipids may enhance bioavailability of poorly soluble drugs *via* a number of potential mechanisms, including (Shah 1994):

1. *Alterations (reduction) in gastric transit:* thereby slowing delivery to the absorption site and increasing the time available for dissolution.

2. *Increase in effective luminal drug solubility:* The presence of lipids in the GI tract stimulates an increase in the secretion of bile salts (BS) and endogenous biliary lipids including phospholipid (PL) and cholesterol (CH), leading to the formation of BS/PL/CH intestinal mixed micelles and an increase in the solubilization capacity of the GI tract. However, intercalation of administered (exogenous) lipids into these BS

Structures either directly (if sufficiently polar), or secondary to digestion, leads to swelling of the micellar structures and a further increase in solubilization capacity

3. *Stimulation of intestinal lymphatic transport:* For highly lipophilic drugs, lipids may enhance the extent of lymphatic transport and increase bioavailability directly or indirectly *via* a reduction in first-pass metabolism.

4. *Changes in the biochemical barrier function of the GI tract:*
It is clear that certain lipids and surfactants may attenuate the activity of intestinal efflux transporters, as indicated by the p-glycoprotein efflux pump, and may also reduce the extent of enterocyte-based metabolism.

5. *Changes in the physical barrier function of the GI tract:*
Various combinations of lipids, lipid digestion products and surfactants have been shown to have permeability enhancing properties . For the most part, however, passive intestinal permeability is not thought to be a major barrier to the bioavailability of the majority of poorly water soluble, and in particular, lipophilic drugs.

6. *Effect of oils on the absorption:*
Such formulations form a fine oil-in-water emulsion with gentile agitation, which may be provided by gastrointestinal motility. A SES also improves the reproducibility of the plasma level–time profile. Various physiological mechanisms have been proposed to explain the effect of oils on the absorption of water-insoluble compounds, including altered gastrointestinal motility, increased bile flow and drug solubilization, increased mucosal permeability, enhanced mesenteric lymph flow, and increased lymphatic absorption of water insoluble drugs and bioavailability also increased of hydrophobic compound.

CHARACTERIZATION OF SDFs:

The primary means of self-emulsification assessment is visual evaluation. The efficiency of self-emulsification could be estimated by determining the rate of emulsification, droplet-size distribution and turbidity measurements (Stegemanna 2007).

1. *Visual assessment*:

This may provide important information about the self emulsifying and microemulsifying property of the mixture and about the resulting dispersion.

2. *Turbidity measurement:*

This is to identify efficient selfemulsification by establishing whether the dispersion reaches equilibrium rapidly and in a reproducible time.

3. *Droplet size:*

This is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as the stability of the emulsion. Photon correlation spectroscopy, microscopic techniques or a coulter nanosizer are mainly used for the determination of the emulsion droplet size. The reduction of the droplet size below 50 µm leads to the formation of SMEDDSs, which are stable, isotropic and clear o/w dispersions. (Kumar 2010)

4. *Zeta potential measurement:*

This is used to identify the charge of the droplets. In conventional SEDDSs, the charge onan oil droplet is negative due to presence of free fatty acids.

5. *Determination of emulsification time:*

Self emulsification time, dispersibility, appearance and flowability was observed and scored according to techniques described in H. Shen et al. used for the grading of formulations.

6. *Refractive Index and Percent Transmittance:*

Refractive index and percent tranmittance proved the transparency of formulation. The refractive index of the system is measured by refractometer by placing drop of solution on slide and it compare with water (1.333)**.** The percent transmittance of the system is measured at particular wavelength using UV-spectrophotometer keeping distilled water as blank. If refractive index of system is similar to the refractive index of water(1.333) and formulation have percent transmittance > 99 percent.

7. *Viscosity Determination:*

The SEDDS system is generally administered in soft gelatin or hard gelatin capsules. So, it can be easily pourable into capsules and such system should not too thick to create a problem. The rheological properties of the micro emulsion are evaluated by Brookfield viscometer. This viscosities determination conform whether the system is w/o or o/w. If system has low viscosity then it is o/w type of the system and if a high viscosity then it is w/o type of the system.

8. *Droplet polarity and droplet size of emulsion:*

Polarity of oil droplets is governed by the HLB value of oil, chain length and degree of unsaturation of the fatty acids, the molecular weight of the hydrophilic portion and concentration of the emulsifier. A combination of small droplets and their appropriate polarity (lower partition coefficient o/w of the drug) permit acceptable rate of release of the drug. Polarity of the oil droplets is also estimated by the oil/water partition coefficient of the lipophillic drug. Size of the emulsion droplet is very important factor in selfemulsification / dispersion performance, since it determine the rate and extent of drug release and absorption. The Coulter nanosizer, which automatically performs photon correlation analysis on scattered light, can be used to provide comparative measure of mean particle size for such system. This instrument detects dynamic changes in laser light scattering intensity, which occurs when particle oscillates due to Brownian movement. This technique is used when particle size range is less than 3µm; a size range for a SMEDDS is 10 to 200 nm .

9. *Dissolution studies:*

For sustained release characteristic, dissolution study is carried out for SEMDDS. Drugs known to be insoluble at acidic pH can be made fully available when it is incorporated in SMEDDS.

10. *Equilibrium phase diagram:*

Although self-emulsification is a dynamic non-equilibrium process involving interfacial phenomena, information can be obtained about self-emulsification using equilibrium phase behavior. There seems to be a correlation between emulsification efficiency and region of enhanced water solubilization and phase inversion region, formation of lamellar liquid crystalline dispersion phase on further incorporation of water. An equilibrium phase diagram enables comparison of different surfactants and their synergy with cosolvent or cosurfactant. The boundaries of one phase region can easily be assessed visually. The phase behavior of a three-component system can be represented by a ternary phase diagram.

11. *Electron microscopic studies:*

Freeze-fracture electron microscopy has been used to study surface characteristics of such dispersed systems. Because of the high liability of the samples and the possibility of artifacts, electron microscopy is considered a somewhat misleading technique. Particle size analysis and low-frequency dielectric spectroscopy have been used to examine the self-emulsifying properties of Imwitor 742 (a mixture of mono- and diglycerides of capric and caprylicacids) and Tween 80 systems .

12. *Cryo-TEM studies:*

For Cryo-Transmission Electron Microscopy (TEM), samples were prepared in a controlled environment verification system. A small amount of sample is put on carbon film supported by a copper grid and blotted by filter paper to obtain thin liquid film on the grid. The grid is quenched in liquid ethane at _1808C and transferred to liquid nitrogen at _1968C. The samples were characterized with a TEM microscope.

13. *Liquefaction time:*

This test is designed to estimate the time required by solid SEDDS to melt in vivo in the absence of agitation to simulated GI conditions. One dosage form is covered in a transparent polyethylene film and tied to the bulb of a thermometer by means of a thread. The thermometer with attached tablets is placed in a round bottom flask containing 250 ml of simulated gastric fluid without pepsin maintained at 37 _ 18C. The time taken for liquefaction is subsequently noted.

14. *Small-angle neutron scattering:*

Small-angle neutron scattering can be used to obtain information on the size and shape of the droplets. The term ‘droplet’ is used to describe micelles, mixed micelles and oil-swollen micelles throughout the present work. Small-angle neutron scattering experiments use the interference effect of wavelets scattered from different materials in a sample (different scattering-length densities).

15. *Small-angle X-ray scattering:*

This a small-angle scattering technique in which the elastic scattering of X-rays by a sample that has non-homogeneities in the nm range is recorded at very low angles (typically 0.1–108). This angular range contains information about the shape and size of macromolecules, characteristic distances of partially ordered materials, pore sizes and other data. Small-angle X-ray scattering is capable of delivering structural information of macromolecules between 5 and 25 nm, of repeat distances in partially ordered systems of up to 150 nm. Small-angle X-ray scattering is used for the determination of the microscale or nanoscale structure of particle systems in terms of such parameters as averaged particle sizes, shapes, distribution and surface-to-volume ratio. The materials can be solid or liquid and they can contain solid, liquid or gaseous domains (so-called ‘particles’) of the same or another material in any combination. In addition to these tools, others – such as nuclear magnetic resonance and differential scanning colorimetry – have also been exploited to characterize these self-emulsifying systems for a better insight.

16. *Thermodynamic stability studies:*

The physical stability of a lipid-based formulation is also crucial to its performance which can be adversely affected by precipitation of the drug in the excipient matrix. In addition, poor formulation physical stability can lead to phase separation of the excipient, affecting not only the formulation performance, but visual appearance as well. In addition, incompatibilities between the formulation and the gelatin capsules shell can lead to brittleness or deformation, delayed disintegration, or incomplete release of drug.

a) Heating cooling cycle: Six cycles between refrigerator temperature (4˚C) and 45˚C with storage at each temperature of not less than 48 h is studied. Those formulations, which are stable at these temperatures, are subjected to centrifugation test.
b) Centrifugation: Passed formulations are centrifuged thaw cycles between 21˚C and

+25°C with storage at each temperature for not less than 48 h is done at 3500 rpm for 30 min. those formulations that do not show any phase separation are taken for the freeze thaw stress test.

c) Freeze thaw cycle: Three freeze for the formulations. Those formulations that passed this test showed good stability with no phase separation, creaming, or cracking.

17. *Dispersibility test:*

The efficiency of self emulsification of oral nano or micro emulsion is assessed using a standard USP XXII dissolution apparatus 2. One milliliter of each formulation was added to 500 ml of water at 37±0.5°C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. The invitro performance of the formulations is visually assessd using the following grading system:

Grade A: Rapidly forming (within 1 min) nanoemulsion, having a clear or bluish appearance.

Grade B: Rapidly forming, slightly less clear emulsion, having a blusih white appearance.

Grade C: Fine milky emulsion that formed within 2 min.

Grade D: Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).

Grade E: Formulation exhibiting either poor or minimal emulsification with large oil globules present on the surface.

17. Electro conductivity study:

The SEDD system contains ionic or non-ionic surfactant, oil, and water so this test is used to measure the electroconductive nature of system. The electro conductivity of resultant system is measured by electroconductometer.

18. In vitro diffusion study:

In vitro diffusion studies are performed to study the release behaviour of formulation from liquid crystalline phase around the droplet using dialysis technique.

19. *Drug content:*

Drug from pre-weighed SEDDS is extracted by dissolving in suitable solvent. Drug content in the solvent extract was analyzed by suitable analytical method against the standard solvent solution of drug.

RECENT ADVANCES IN SDFs:

1. Dry emulsion
2. Self emulsifying capsules
3. Self emulsifying sustained/controlled release tablets
4. Self emulsifying sustained/controlled release pellets
5. Self emulsifying solid dispersions
6. Self emulsifying sustained release microspheres
7. Self emulsifying nanoparticles
8. Self emulsifying suppositories
9. Self emulsifying implants (Tatyana and Benita 2000)
10. Micro emulsion drug delivery

SOLID SELF-MICROEMULSIFYING DRUG DELIVERY SYSTEM (S-SMEDDS):

SMEDDS can exist in either liquid or solid states. SMEDDS are usually, limited to liquid dosage forms, because many excipients used in SMEDDS are not solids at room temperature. Given the advantages of solid dosage forms, S-SMEDDS have been extensively exploited in recent years, as they frequently represent more effective alternatives to conventional liquid SMEDDS. From the perspective of dosage forms, SSMEDDS mean solid dosage forms with self-emulsification properties. S-SMEDDS focus on the incorporation of liquid/semisolid SE ingredients into powders/ nanoparticles by different solidification techniques (e.g. adsorptions to solid carriers, spray drying, melt extrusion, nanoparticles technology, and so on). Such powders/nanoparticles, which refer to SE nanoparticles/dry emulsions/solid dispersions are usually further processed into other solid SE dosage forms, or, alternatively, filled into capsules (i.e. SE capsules). SE capsules also include those capsules into which liquid/semisolid SEDDS are directly filled without any solidifying excipient. (al 2008)

SOLIDIFICATION TECHNIQUES FOR TRANSFORMING LIQUID/SEMISOLID SMEDDS TO S-SMEDDS:

Various solidification techniques are listed below:

1. Capsule filling with liquid and semisolid self emulsifying formulations
2. Spray drying
3. Adsorption to solid carriers
4. Melt granulation
5. Melt extrusion/ Extrusion spheronization (Bo 2008)

APPLICATIONS OF SDFs:

- ### *Improvement in Solubility and bioavailability:*

If drug is incorporated in SEDDS, it increases the solubility because it circumvents the dissolution step in case of Class-Π drug (Low solubility/high permeability). Ketoprofen, a moderately hydrophobic (log P 0.979) nonsteroidal anti-inflammatory drug (NSAID), is a drug of choice for sustained release formulation has high potential for gastric irritation during chronic therapy. Also because of its low solubility, ketoprofen shows incomplete release from sustained release formulations. Vergote et al. (2001) reported complete drug release from sustained release formulations containing ketoprofen in nanocrystalline form Different formulation approaches that have been sought to achieve sustained release, increase the bioavailability, and decrease the gastric irritation of ketoprofen include preparation of matrix pellets of nano-crystalline ketoprofen, sustained release ketoprofen microparticles and formulations, floating oral ketoprofen systems, and transdermal systems of ketoprofen. (Pal 2011)

- ### *Protection against Biodegradation:*

The ability of self emulsifying drug delivery system to reduce degradation as well as improve absorption may be especially useful for drugs, for which both low solubility and degr (Kyatanwar 2010)adation in the GI tract contribute to a low oral bioavailability. Many drugs are degraded in physiological system, may be because of acidic pH in stomach, enzymatic degradation or hydrolytic degradation etc.

FUTURE TRENDS:

In relation to formulation of poorly soluble drugs in the future, there are now techniques being use to convert liquid/ semi solid SEDDS and SMEDDS formulations into powders and granules, which can then be further processed into conventional 'powder fill' capsules or even compressed into tablets. Hot melt granulation is a technique for producing granules or pellets, and by using a waxy solubilising agent as a binding agent, upto 25% solubilising agent can be incorporated in a formulation. There is also increasing interest in using inert adsorbents, such as the Neusilin (Fuji Chemicals) and Zeopharm (Huber) products for converting liquids into powders- which can be processed into powder fill capsules or tablets.

But to obtain solids with suitable processing properties, the ratio of SEDDS to solidifying excipients must be very high, which seems to be practically non-feasible for drugs having limited solubility in oil phase. In this regard, it was hypothesized that the amount of solidifying excipients required for transformation of SEDDS in solid dosage forms will be significantly reduced if SEDDS is gelled. Colloidal silicon dioxide (Aerosil 200) is selected as a gelling agent for the oil based systems, which may serve the dual purpose of reducing the amount of solidifying excipients required and aiding in slowing drug release.

Drug name	Compound	Dosage form	Company	Indication
Neoral®	Cyclosporine A/I	SGC	Novartis	Immuno suppressant
Fortovase ®	Saquinavir	SGC	Hoffmann-La Roche Inc.	HIV antiviral
Convulex ®	Valproic acid	SGC	Pharmacia	Antiepileptic
Targretin ®	Bexarotene	SGC	Ligand	Antineoplastic
Gengraf ®	Cyclosporine A/III	HGC	Abbott laboratories	Immune suppressant

Table 1: Examples of marketed SEDDS formulations

SGC: Soft Gelatin Capsules

HGC: Hard Gelatin Capsules

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INTRODUCTION TO BIOADHESION/MUCOADHESION

Kamble Pranay, Katedeshmukh Ramesh

The term bioadhesion refers to any bond formed between two biological surfaces or a bond between a biological and a synthetic surface. In case of bioadhesive drug delivery, the term bioadhesion is used to describe the adhesion between polymers, either synthetic or natural and soft tissues or the gastrointestinal mucosa. In cases where the bond is formed with the mucus the term mucoadhesion may be used synonymously with bioadhesion. Mucoadhesion can be defined as a state in which two components, of which one is of biological origin, are held together for extended periods of time by the help of interfacial forces. Generally speaking, bioadhesion is a term which broadly includes adhesive interactions with any biological or biologically derived substance, and mucoadhesion is used when the bond is formed with a mucosal surface.

Concepts of Buccal Drug Delivery System: (www.authorstream.com 2012)

Mucoadhesive polymers as drug delivery vehicles. The common principle underlying this drug administration route is the adhesion of the dosage form to the mucous layer until the polymer dissolves or the mucin replaces itself. Benefits for this route of drug administration are: prolonged drug delivery, targeted therapy and often improved bioavailability.

Mechanism Of Mucoadhesion: (Sanket D. Gandhi July-2011)

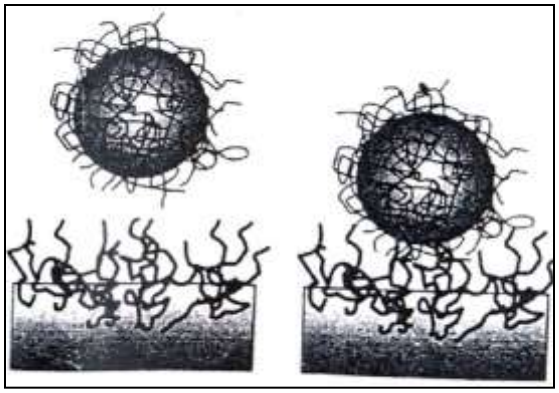


Fig- 1 mechanism of adhesion (Sanket D. Gandhi July-2011)

As stated, mucoadhesion is the attachment of the drug along with a suitable carrier to the mucous membrane. Mucoadhesion is a complex phenomenon which involves wetting, adsorption and interpenetration of polymer chains. Mucoadhesion has the following mechanism:

1. Intimate contact between a bioadhesive and a membrane (wetting or swelling phenomenon).
2. Penetration of the bioadhesive into the tissue or into the surface of the mucous membrane (interpenetration). Residence time for most mucosal routes is less than an hour and typically in minutes, it can be increased by the addition of an adhesive agent in the delivery system which is useful to localize the delivery system and increases the contact time at the site of absorption. The exact mechanism of mucoadhesion is not known but an accepted theory states that a close contact between the mucoadhesive polymer and mucin occurs which is followed by the interpenetration of polymer and mucin. The adhesion is prolonged due to the formation of van der vaals forces, hydrogen bonds and electrostatic bonds.

Theories of mucoadhesion: (Andrews GP *et al.*, 2000)

Wettability theory
Electronic theory
Fracture theory
Adsorption theory
Diffusion theory

Wettability theory:

The ability of bioadhesive or mucus to spread and develop intimate contact with its corresponding substrate is an important factor in bond formation. The wetting theory was developed predominantly in regard to liquid adhesives, uses interfacial tensions to predict spreading and in turn adhesion. The study of surface energy of polymers and tissues to predict mucoadhesive performance has been given considerable attention.

The contact angle (Q) which should ideally be zero for adequate spreading is related to interfacial tensions (g) as per the Youngs equation,

g tg = g bt + gbg cos Q

Where the subscripts t,g and b represent tissue, gastrointestinal contents and bioadhesive polymer respectively, for spontaneous wetting to occur

gtb ≥ gbt + gbg

The spreading coefficient, Sb/t can be given by,

Sb/t = gtg - gbt - gbg

For the bioadhesion to take place the spreading coefficient must be positive, hence it is advantageous to maximize the interfacial tension at the tissue-GI contents interface and minimizing the surface tension at the other two interfaces. The interfacial tension can be measured by methods like the Wilhel my plate method. It has been shown that the BG-tissue interfacial tension can be calculated as,

g bt = gb + gt – 2F(gbgt)1/2

` parameter) can be found in published papers thus by the wetting theory it is possible to calculate spreading coefficients for various bioadhesives over biological tissues and predict the intensity of the bioadhesive bond.

Electronic theory:

The electronic theory depends on the assumption that the bioadhesive material and the target biological material have different electronic surface characteristics. Based on this, when two surfaces come in contact with each other, electron transfer occurs in an attempt to balance the Fermi levels, resulting in the formation of a double layer of electrical charge at the interface of the bioadhesive and the biologic surface. The bioadhesive force is believed to be present due to the attractive forces across this double layer.

Fracture theory:

This is by-far the most accepted theory on bioadhesion. It explains the forces required to separate the two surfaces after adhesion has taken place. It measures the maximum Tensile stress(sm) produced during detachment as follows

s m = Fm/Ao

Where Fm and Ao represent the maximum force of detachment and the total surface area respectively. In a uniform single-component system, fracture strength (sf), which is equal to the maximum stress of detachment(sm), is proportional to the fracture energy (gc), Youngs modulus of elasticity (E) and the critical crack length (c) of the fracture site as follows,

s f = (gcE/c)1/2

fracture energy can be obtained by the sum of the reversible work of adhesion, Wr (work done to produce new fracture surfaces) and the irreversible work of adhesion, Wi (work of plastic deformation),

g c = Wr + Wi

Adsorption theory:

This theory states that the bioadhesive bond formed between an adhesive substrate and the tissue is due to the weak van der waals forces and hydrogen bond formation. It is one of the most widely accepted theories of bioadhesion.

Diffusion theory:

The concept of the interpenetration and entanglement of the bioadhesive polymer chains and mucous polymer chains is supported by the diffusion theory. The bond strength increases with the increase in the degree of the penetration. This penetration is dependent on the concentration gradients and the diffusion coefficients. It is believed that interpenetration in the range of 0.2-0.5μm is required to produce effective bond strength. The penetration depth can be estimated by

l = (tDb) 1/2

Where t is the time of contact and Db is the diffusion coefficient of the bio adhesive material in the mucus.

Factors affecting mucoadhesion: (Pranshu Tangri 2011) (Ch’ng HS 1985)

The mucoadhesion of a drug carrier system to the mucous membrane depends on the below mentioned factors.

- Polymer based factors- Molecular weight of the polymer, Concentration of polymer used, Flexibility of polymer chains, Swelling factor, Stereochemistry of polymer.
- Physical factors- pH at polymer substrate interface, Applied strength, Contact time.
- Physiological factors -Mucin, turnover rate, Diseased state.

Types of Mucosa:

(www.authorstream.com 2012)

1. Buccal mucosa
2. Esophageal mucosa
3. Gastric mucosa

4. Intestinal mucosa
5. Nasal mucosa
6. Olfactory mucosa
7. Oral mucosa
8. Bronchial mucosa
9. Uterine mucosa

Anatomy And Nature Of Oral Cavity:

(Silvia Rossi 2005) (Sanket D. Gandhi July-2011)

The oral cavity may be divided into two regions, the outer oral vestibule, bounded by the lips and cheeks and the oral cavity itself the borders being, and formed by the hard and soft palates, the floor of the mouth and tonsils.

Physical Description of Oral Cavity:

The mucosa that lines the oral cavity may be divided into three types, classified according to their function as;

1. Masticatory mucosa: Which includes the mucosa around the teeth and on the hard palate and these regions have keratinized epithelium.

2. Lining mucosa**:** Which covers the lips, cheeks, fornix, base of the oral cavity, lower part of tongue, buccal mucosa and the soft palate and these regions have nonkeratinized epithelium.

3. Specialized mucosa: covering the dorsum of the tongue with highly keratinization.

Oral mucosa:

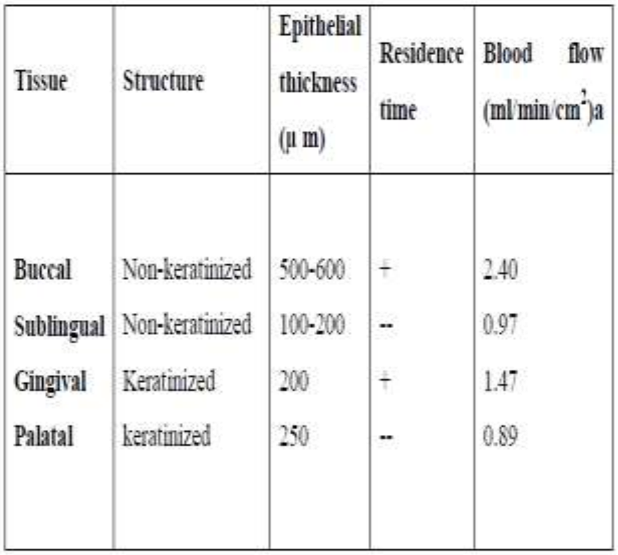
The oral mucosa is composed of an outermost layer of stratified squamous epithelium (about 40-50 cell layers thick), a lamina propria followed by the submucosa as the innermost layer (Figure-2).

The composition of the epithelium varies depending on the site in the oral cavity.

The mucosae of the gingivae and hard palate are keratinized similar to the epidermis contain neutral lipids like ceramides and acylceramides which are relatively impermeable to water. The mucosae of the soft palate, the sublingual, and the buccal regions, however, are not keratinized contain only small amounts of ceramide.

Regional variation in the composition of oral mucosa: (Sanket D. Gandhi July-2011) (Harris D 1992)

The membrane that lines the oral cavity has a total area of 200 cm2 and shows difference in structures thickness and blood flow depending on their location. Keratinized and non- keratinized tissue occupies about 50% and 30% respectively.



Tissue	Structure	Epithelial thickness (µ m)	Residence time	Blood flow (ml/min/cm^2)a
Buccal	Non-keratinized	500-600	+	2.40
Sublingual	Non-keratinized	100-200	--	0.97
Gingival	Keratinized	200	+	1.47
Palatal	keratinized	250	--	0.89

Table 1: Regional variation in the composition of oral mucosa

Composition of Mucus Layer: (Harris D 1992)

Mucus is a translucent and viscid secretion which forms a thin, contentious gel, mean thickness of this layer varies from about 50-450 µm in humans secreted by the globet cells lining the epithelia or by special exoesive glands with mucus cell acini. It has the following general composition.

- Water -95%
- Glycoprotein and lipids – 0.5-3.00%
- Mineral salts – 1%
- Free proteins – 0.5-1.0%

Schematic diagram of buccal mucosa:

(Pankil A. Gandhi 2011) (H 1998) (Miller N.S. 2005)

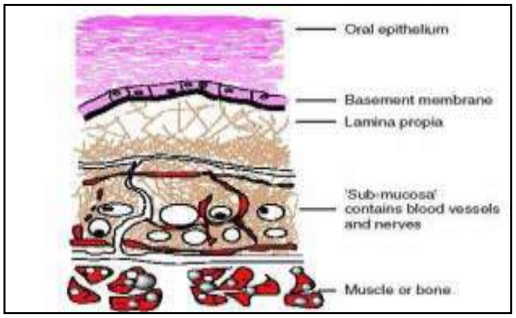


Figure 2: Buccal Mucosa

Functions of Mucus Layer: (Sanket D. Gandhi July-2011)

1. Protective: resulting particularly from its hydrophobicity.
2. Barrier: The role of the mucus layer as a barrier in tissue absorption of the drugs and influence the bioavailability.
3. Adhesion: Mucus has strong cohesion properties.
4. Lubrication: It is to keep the mucus from the goblet cell is necessary to compensate for the removal of the mucus layer due to digestion, bacterial degradation and solubilization of mucin molecules

Salivary secretion:

There are mainly three glands which secrets saliva in the oral cavity, parotid, sublingual and sub-mandibular. Functions of saliva are Moisten the oral cavity, aid the digestion of foods, lubricate the food for mastication and swallowing, provide protection to the tissue from abrasion by rough Reduction in dose can be achieved, thereby reducing dose dependent side effects.

Advantages of mucoadhesive buccal drug delivery: (Sanket D. Gandhi July-2011)

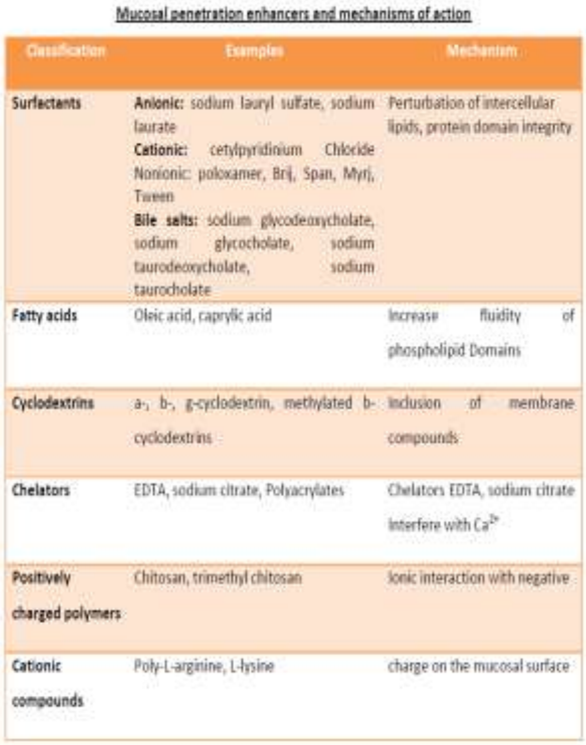
1. It allows local modification of tissue permeability, inhibition of protease activity or reduction in immunogenic response, thus selective use of therapeutic agents like peptides, proteins and ionized species can be achieved.
2. Drugs which are unstable in acidic environment of stomach or destroyed by the alkaline environment of intestine can be given by this route
3. Drugs which show poor bioavailability by oral route can be administered by this route.
4. It follows passive diffusion, and does not require any activation.
5. The presence of saliva ensures large amount of water for dissolution of drug unlike in case of rectal and transdermal route.
6. Drugs with short half life can be administered by this method. (2-8 hrs) e.g. :- nitroglycerine (2 hrs) isosorbide mononitrate (2-5 hrs)
7. From the formulation point of view a thin mucin film exist on the surface of oral cavity.
8. Provides opportunity to retain delivery system in contact with mucosa for prolonged period of time with the help of mucoadhesive compounds.
9. The buccal membrane is sufficiently large to allow delivery system to be placed at different sites on the same membrane for different occasions, if the drug or other excipients cause reversible damage or irritate mucosa.
10. Easy of administration and termination of therapy in emergency.
11. Permits localization of the drug for a prolonged period of time.
12. Can be administered to unconscious and trauma patients.
13. Offers an excellent route for the systemic delivery of drug which by passes first pass
14. Metabolism, thereby offering a greater bioavailability.
15. Significant reduction in dose can be achieved; thereby reducing dose, dose dependentside effects, and eliminates peak-valley profile.

Disadvantages of Buccal Drug Delivery Systems

1. Over hydration may lead to formation of slippery surface & structural integrity of the formulation may get disrupted by the swelling & hydration of the bioadhesive polymer.
2. Eating and drinking may become restricted
3. There is possibility that Patient may swallow the tablet
4. The drug contained in swallowed saliva follows the per oral route & advantages of buccal route is lost.
5. Only drug with small dose requirement can be administered.
6. Drug which irritate mucosa or have a bitter or unpleasant taste or an obnoxious odour cannot be administered by this route
7. Drugs which are unstable at buccal pH cannot be administered by this route.

8. Only those drugs which are absorbed by passive diffusion can be administered by this route

Permeability Enhancers/ oral mucosal permeation enhancer: (Kumar V JULY-SEPT 2011)



Mucosal penetration enhancers and mechanisms of action

Classification	Examples	Mechanism
Surfactants	**Anionic:** sodium lauryl sulfate, sodium laurate **Cationic:** cetylpyridinium Chloride Nonionic: poloxamer, Brij, Span, Myrj, Tween **Bile salts:** sodium glycodeoxycholate, sodium glycocholate, sodium taurodeoxycholate, sodium taurocholate	Perturbation of intercellular lipids, protein domain integrity
Fatty acids	Oleic acid, caprylic acid	Increase fluidity of phospholipid Domains
Cyclodextrins	a-, b-, g-cyclodextrin, methylated b-cyclodextrins	Inclusion of membrane compounds
Chelators	EDTA, sodium citrate, Polyacrylates	Chelators EDTA, sodium citrate Interfere with Ca^{2+}
Positively charged polymers	Chitosan, trimethyl chitosan	Ionic interaction with negative
Cationic compounds	Poly-L-arginine, L-lysine	charge on the mucosal surface

Table-2 Permeability Enhancers

Ideal Drug Candidates for Buccal Drug Delivery System: (www.authorstream.com 2012)

- Molecular size – 75-600 daltons
- Molecular weight between 200-500 daltons.
- Drug should be lipophilic or hydrophilic in nature.
- Stable at buccal pH.
- Taste – bland
- Drug should be odourless.
- Drugs which are absorbed only by passive diffusion should be used

Methods to Increase Drug Delivery via Buccal Route (RAJESH MUJORIYA 2011)

(1) Absorption enhancers

Absorption enhancers have demonstrated their effectiveness in delivering high molecular weight compounds, such as peptides, that generally exhibit low buccal absorption rates.

(2) Prodrugs

Hussein et al delivered opioid agonists and antagonists in bitterness prodrug forms and found that the drug exhibited low bioavailability as prodrug Nalbuphine and naloxone bitter drugs when administered to dogs via the buccal mucosa, the caused excess salivation and swallowing. As a result, the drug exhibited low bioavailability.

(3) pH: Shojaei et al evaluated permeability of acyclovir at pH ranges of 3.3 to 8.8, and in the presence of the absorption enhancer, sodium glycocholate. The in vitro permeability of acyclovir was found to be pH dependent with an increase in flux and permeability coefficient at both pH extremes (pH 3.3 and 8.8), as compared to the mid-range values (pH 4.1, 5.8, and 7.0).

(4) Patch design:

Several in vitro studies have been conducted regarding on the type and amount of backing materials and the drug release profile and it showed that both are interrelated. Also, the drug release pattern was different between single-layered and multi-layered patches

Classification Of Buccal Bioadhesive Dosage Forms: (RAJESH MUJORIYA 2011)

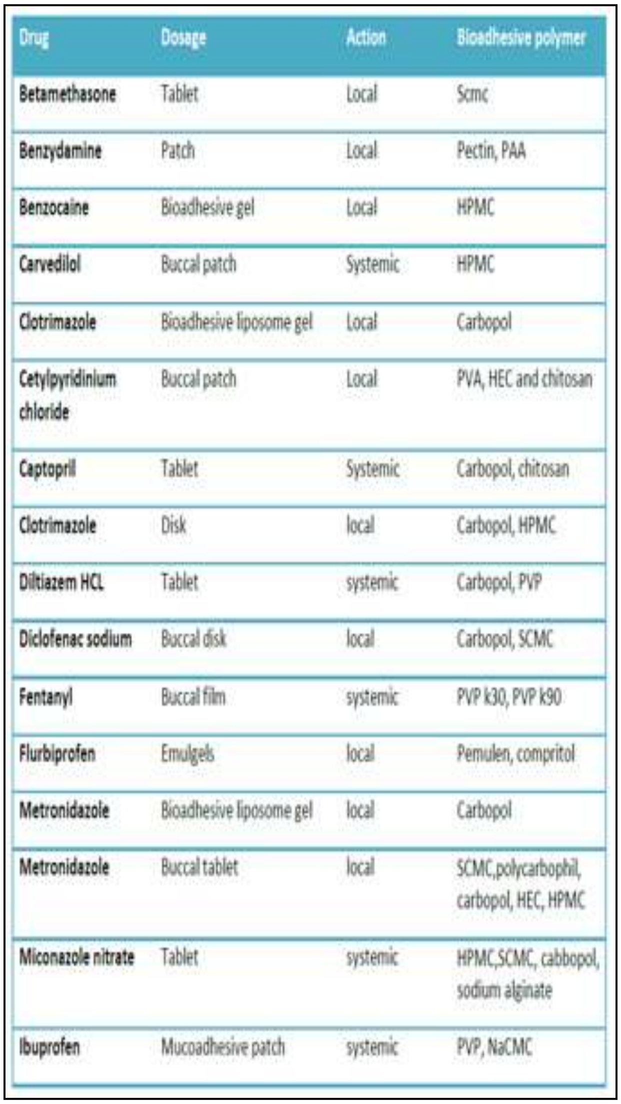
In the development of these Buccal drug delivery systems, mucoadhesion of the device is a key element. The term 'mucoadhesive' is commonly used for materials that bind to the mucin layer of a biological membrane. Mucoadhesive polymers have been utilized in many different dosage forms in efforts to achieve systemic delivery of drugs through the different mucosa. These dosage forms include tablets, patches, tapes, films, semisolids and powders. To serve as mucoadhesive polymers, the polymers should possess some general physiochemical features such as

I. Predominantly anionic hydrophilicity with numerous hydrogen bond-forming groups

II. Suitable surface property for wetting mucus/mucosal tissue surfaces

III. Sufficient flexibility to penetrate the mucus network or tissue crevices

The polymers which have been tried and tested over the years include Carbopol, Polycarbophil, Poly(acrylicacid/ divinyl benzene), Sodium Alginate, Hydroxyethyl cellulose, Hydroxypropyl methylcellulose, Hyaluronic acid, Gelatin, Guar Gum, Thermally modified Starch, Pectin, Polyvinyl pyrrolidone, Acacia, Polyethylene Carboxymethyl cellulose, glycol, Psyllium Amberlite-200 resin, Hydroxypropyl cellulose, Chitosan, Hydroxyethyl methacrylate. There are some Novel Mucoadhesive Polymers under development **,** these include Copolymer of PAA and PEG monoethylether monomethacrylate, PAA complexed with PEGylated drug conjugate, Hydrophilic pressure-sensitive adhesives (PSAs), AB block copolymer of oligo(methyl methacrylate) and PAA , Polymers with thiol groups (cysteine was attached covalently to polycarbophil by using carbodiimide as a media.



Drug	Dosage	Action	Bioadhesive polymer
Betamethasone	Tablet	Local	Scmc
Benzydamine	Patch	Local	Pectin, PAA
Benzocaine	Bioadhesive gel	Local	HPMC
Carvedilol	Buccal patch	Systemic	HPMC
Clotrimazole	Bioadhesive liposome gel	Local	Carbopol
Cetylpyridinium chloride	Buccal patch	Local	PVA, HEC and chitosan
Captopril	Tablet	Systemic	Carbopol, chitosan
Clotrimazole	Disk	local	Carbopol, HPMC
Diltiazem HCL	Tablet	systemic	Carbopol, PVP
Diclofenac sodium	Buccal disk	local	Carbopol, SCMC
Fentanyl	Buccal film	systemic	PVP k30, PVP k90
Flurbiprofen	Emulgels	local	Pemulen, compritol
Metronidazole	Bioadhesive liposome gel	local	Carbopol
Metronidazole	Buccal tablet	local	SCMC,polycarbophil, carbopol, HEC, HPMC
Miconazole nitrate	Tablet	systemic	HPMC,SCMC, cabbopol, sodium alginate
Ibuprofen	Mucoadhesive patch	systemic	PVP, NaCMC

Table-3 Reported Buccoadhesive Drug Delivery System (Kumar V* JULY-SEPT 2011)

Current Status Of Buccal Bioadhesive Dosage Form: (Patel K.V. 2011)

Dosage forms such as mouthwashes, erodible/ chewable buccal tablets, and chewing gums allow only a short period of release, and reproducibility of drug absorption is poor. Application of bioadhesive semisolid gels creates considerable technical problems. Bioadhesive buccal films/patches and tablets are the less developed type of dosage forms. These bioadhesive buccal films/patches and tablets were usually fabricated in different geometry, as shown in Fig. A. Type I is a single-layer device, from which drug can be released multi directionally. Type II device has a impermeable backing layer on top of the drug-loaded bioadhesive layer, and drug loss into oral cavity can be greatly decreased. Type III is a unidirectional release device, from which drug loss will be avoided and drug can penetrate only via the buccal mucosa.

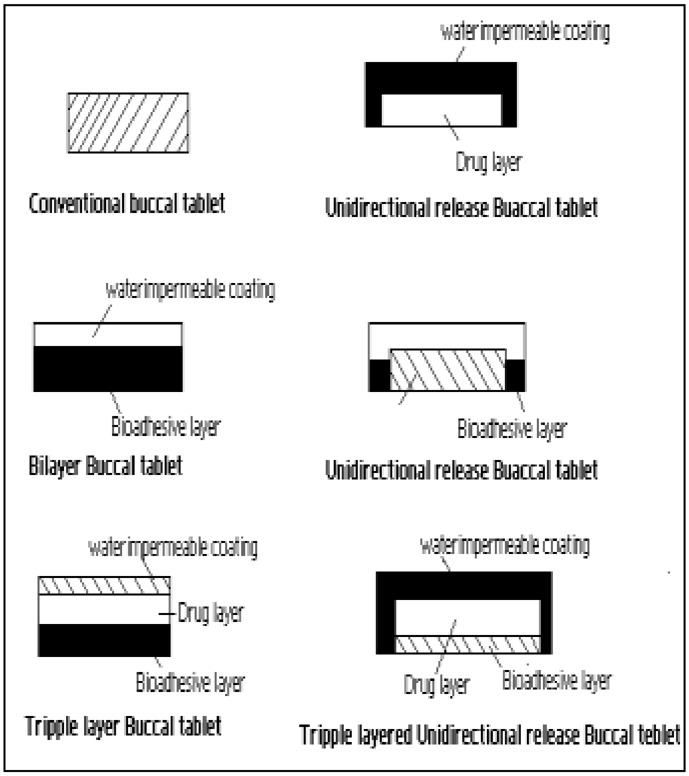
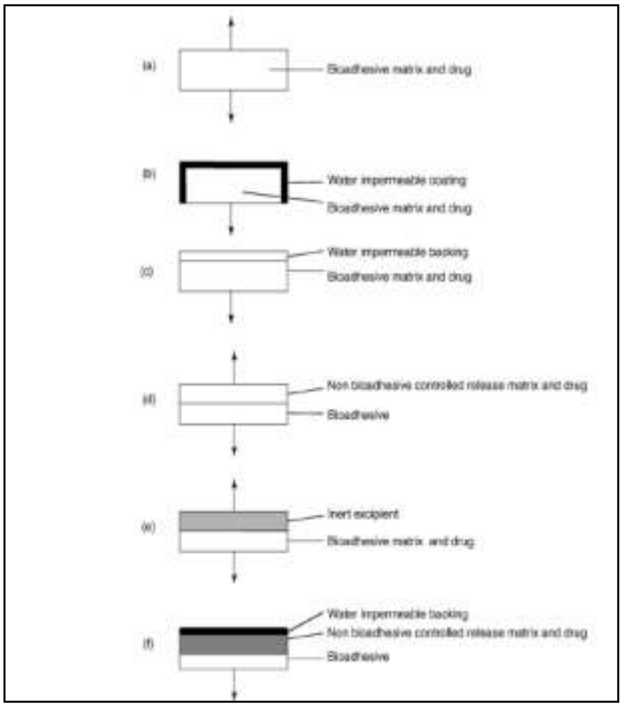

water impermeable coating
Drug layer
Conventional buccal tablet
Unidirectional release Buaccal tablet
water impermeable coating
Bioadhesive layer
Bilayer Buccal tablet
Bioadhesive layer
Unidirectional release Buaccal tablet
water impermeable coating
Drug layer
Bioadhesive layer
Tripple layer Buccal tablet
water impermeable coating
Drug layer
Bioadhesive layer
Tripple layered Unidirectional release Buccal teblet

Figure-2 Tablet Forms Use In Buccal Drug Delivery System (Patel K.V. 2011)

1. Matrix type: The buccal patch designed in a matrix configuration contains drug, adhesive, and additives mixed together.


(a)
Bioadhesive matrix and drug
(b)
Water impermeable coating
Bioadhesive matrix and drug
(c)
Water impermeable backing
Bioadhesive matrix and drug
(d)
Non bioadhesive controlled release matrix and drug
Bioadhesive
(e)
Inert excipient
Bioadhesive matrix and drug
(f)
Water impermeable backing
Non bioadhesive controlled release matrix and drug
Bioadhesive

Schematic representation of matrix tablet arrow direction shows drug release of tablet

2. Reserviour type: The buccal patch designed in a reservoir system contains a cavity for the drug and additives separate from the adhesive. An impermeable backing is applied to control the direction of drug delivery; to reduce patch deformation and disintegration while in the mouth; and to prevent drug loss. Additionally, the patch can be constructed to undergo minimal degradation in the mouth, or can be designed to dissolve almost immediately.

Transmucosal drug delivery systems can be bi-directional or unidirectional. Bi-directional (Figure 3. patches release drug in both the mucosa and the mouth while, Unidirectional (Figure 4) patches release the drug only into the mucosa.

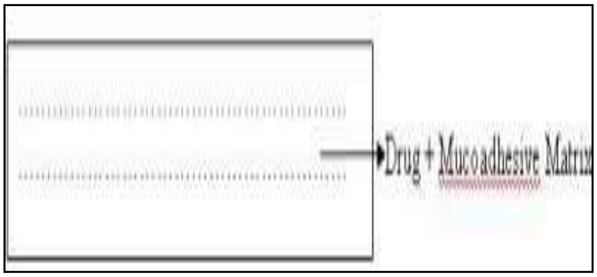


Figure 3: Buccal Patch designed for Bidirectional drug release (Patel K.V. 2011)

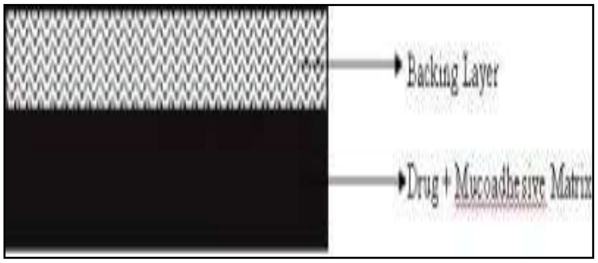


Figure 4: Buccal Patch designed for unidirectional drug release (Patel K.V. 2011)

The adhesive part of the system can be used as drug carrier or as an adhesive for the retention of a drug loaded non-adhesive layer. The use of as an impermeable backing layer will maximize the drug concentration gradient and prolong adhesion because the system is protected from saliva.

Bioadhesive films/patches are commonly manufactured by solvent casting methods using adhesive coating machines, which involve dissolving a drug in a casting solution, casting film, and drying and laminating with a backing layer or a release liner. The processing technology is quite similar to pressure sensitive adhesive-based patch manufacturing. Very recently, a hot-melt extrusion method was reported to fabricate hot-melt extruded films for buccal delivery, which overcomes the disadvantages associated with a solvent casting method such as environmental concerns, long processing times, and high costs.

Buccal chewing gums: (Patel K.V. 2011)

Although medicated chewing gums pose difficulties in regulating the dose administered, they still have some advantages as drug delivery devices, particularly in the treatment of diseases in the oral cavity and in nicotine replacement therapy. Some commercial products are available in the market. Caffeine chewing gum, Stay Alert®, was developed recently for alleviation of sleepiness. It is absorbed at a significantly faster rate and its bioavailability was comparable to that in capsule formulation. Nicotine chewing gums (e.g., Nicorette® and Nicotinell®) have been marketed for smoking cessation. The permeability of nicotine across the buccal mucosa is faster than across the skin. However, chewing gum slowly generates a steady plasma level of nicotine rather than a sharp peak as experienced when smoking. Possible swallowing of considerable amount of nicotine during chewing may lead to decreased effectiveness of the chewing gum due to first-pass metabolism and gastrointestinal discomfort. It is a major challenge to optimize the dose-response relationship of nicotine administered in a chewing gum.

Bioadhesive hydrogel tablets: (Patel K.V. 2011)
Bioadhesive tablets comprising hydrogels can adhere to the buccal mucosa. They are similar to conventional tablets and are prepared by wet granulation, dry granulation, or direct compression processes. Drug is released upon the hydration and adhesion of the device. Buccal tablets should be fabricated and optimized for swelling behavior and drug release to ensure a prolonged period of bioadhesion and sustained or controlled release. Generally, the tablets are formulated with flat punches with dimensions less than 10 mm in diameter and 2 mm thick to aid in establishing intimate contact with buccal mucosa and reduce their interference with normal activities. In addition to mucoadhesive components, most of the tablets contained water-soluble excipients such as high-molecular-weight polyethylene glycols and mannitol. A single-layer buccal tablet of triamcinolone acetonide, Aftach®, is used in the treatment of aphthous ulcers

Bilayer buccoadhesive tablets: (Patel K.V. 2011)
Specialized tablet formulations with two layers are being designed to promote unidirectional drug absorption, minimize drug leakage into buccal cavity, and to achieve biphasic drug release. Iga and Ogawa formulated a slowly disintegrating gingival tablet for sustained release of isosorbide dinitrate and nitroglycerin. Flatfaced tablets 8 mm in diameter were prepared using lactose and hydroxypropyl cellulose. In order to control the deformation of the tablet caused by softening and mouth movements, they were covered with a bioadhesive containing polyethylene film with a 5 mm hole in the center of the top surface. When evaluated in dogs, these tablets remained in position for about 10 hours, whereas plain tablets disintegrated within 3–6 hours. Constant blood drug levels were maintained for about 10 hours from covered tablets. It has been shown that the rate of tablet disintegration, which in turn refers the buccal residence and the drug blood levels, can be controlled by changing the size of hole. A size larger than 50% of the top surface of tablets is suggested to obtain a constant disintegration rate

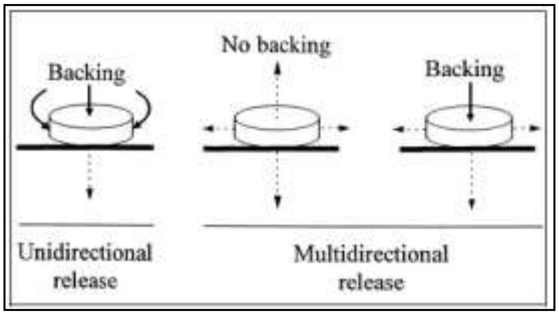


Figure-5: Schematic representation of Unidirectional and Bidirectional release from buccal Tablet

Recent Innovations Related To Dosage Forms: (Patel K.V. 2011)

1. Biobadhesive Spray:
Buccoadhesive sprays are gaining popularity over other dosage forms because of flexibility, comfort, high surface area and availability of drug in solution form. The fentanyl Oralet ™ is the first FDA-approved (1996) formulation developed to take advantage of oral transmucosal absorption for the painless administration of an opioid in a formulation acceptable to children. In 2002, the FDA approved Subutex (buprenorphine) for initiating treatment of opioid dependence (addiction to opioid drugs, including heroin and opioid analgesics) and Suboxone (buprenorphine and naloxone) for continuing treatment of addicts. In 2005, Oral-lyn buccal spray was approved for commercial marketing and sales in Ecuador.

2. Gel Forming Liquids:
This type of a formulation is liquid upon instillation and undergoes a phase transition to form a viscoelastic gel in response to stimulus such as temperature, ionic strength or pH. Carbomers become more viscous upon increased pH. Poloxamers and smart hydrogel®(Adnaced medical solution) gel at approximately body temperature. Gellan gum and alginate both form gel in response to increased ionic strength (particularly with Ca^{+2} ions). Gel forming formulations are currently used for sustained ocular delivery. Recent work has examined the oesophageal retention of smart Hydrogel®, a liquid that gels in

response to both high force and temperature, with its gelling temperature at about 32°C

3. Thiolated tablet:

Another obstacle for delivering peptides across the buccal mucosa is the proteolytic hydrolysis of peptidic molecules. However, there are few proteolytic enzymes as compared to oral, nasal, vaginal and rectal administration. Indeed, the buccal mucosa seems to be deficient in proteinases such as pepsin, trypsin and chymotrypsin present in gastric and intestinal secretion which are known to contribute to peptide hydrolysis. Aminopeptidases appear to be the only peptidases active on the buccal mucosa, therefore representing a major metabolic barrier to the buccal delivery of peptide drugs. The absence of endopeptidase and carboxypeptidase activities will be advantageous for the buccal delivery of peptides which are susceptible to these activities. Recently, it could be observed that thiolation of polycarbophil (PCP) enhances the inhibitory potency of PCP towards aminopeptidase N and membrane bound peptidases involved in the digestion of leu-p-nitroanilide (leu-pNA) and leucin-enkephalin (leu-enkephalin). A combination of different properties within a unique system, for instance mucoadhesive and enzyme inhibiting properties could be obtained by the use of thiolated PCP in a buccal drug delivery system. The covalent attachment of cysteine to the anionic polymer PCP leads to an improvement of the stability of matrix-tablets consisting of thiolated polymer. The mucoadhesive properties are also enhanced, which is confirmed by two different in vitro test systems. In addition, thiolation increases the inhibitory potency of PCP towards buccal enzymes, and thereby the stability of leu-enekphalin and leu-pNA is raised. Due to these features matrix-tablets based on thiolated PCP represent a promising type of buccal drug delivery systems.

Limitation of Buccal Drug Administration (Pankil A. Gandhi 2011)

Drug administration via buccal mucosa has certain limitations.

1. Drugs, which irritate the oral mucosa, have a bitter or unpleasant taste, odour; can not be administered by this route.
2. Drugs, which are unstable at buccal pH can not be administered by this route.
3. Only drugs with small dose requirements can be administered.
4. Drugs may swallow with saliva and loses the advantages of buccal route.
5. Only those drugs, which are absorbed by passive diffusion, can be administered by this route.
6. Eating and drinking may become restricted.
7. Swallowing of the formulation by the patient may be possible.
8. Over hydration may lead to the formation of slippery surface and structural integrity of the formulation may get disrupted by the swelling and hydration of the bioadhesive polymers.

Methods of evaluation: (Pankil A. Gandhi 2011)

Mucoadhesive polymers can be evaluated by testing their adhesion strength by both in vitro and in vivo tests.

***In vitro* tests / exvivo.**

The importance is layed on the elucidation of the exact mechanisms of bioadhesion. These methods are,

- Methods determining tensile strength - fluorescent probe method
- flow channel method
- Mechanical spectroscopic method
- falling liquid film method
- Colloidal gold staining method
- Viscometer method
- thumb method
- Adhesion number
- Electrical conductance
- swelling properties
- In vitro drug release studies
- Mucoretentability studies

***In vivo* methods.**

- Use of radioisotopes
- Use of gamma scintigraphy
- Use of pharmacoscintigraphy
- Use of electron paramagnetic resonance (EPR) oximetry
- X ray studies
- Isolated loop technique
- Methods determining shear stress
- Adhesion weight method

***In vitro/Ex vivo* methods: Methods based on measurement of tensile strength:** (Kumar V, Buccal Bioadhesive Drug Delivery- A Novel Technique 2011)

In these methods the force required to break the adhesive bond between a model membrane and the test polymer is measured.

Tensinometer:

This instrument consists of two jaws from flat glasses. The upper glass was fixed, but the lower glass had been mounted on a screw-elevating surface. The upper fixed glass was attached to a sensitive digital balance. Tablets from each formulations were suspended in water (pH 7) for 15 min. Then these adhesive tablets were located on the surface of lower glass and were elevated until they contact the surface of upper glass. The lower glass was then lowered until the tablet clearly was pulled free from the upper glass. The maximum tensile force needed to detach the jaws was recorded in gram/cm and mean values were calculated and recorded.

Modified balance method:

Modified double beam physical balance was used as the Bioadhesion test apparatus. The right pan of the balance was replaced with lighter one and pan was prepared with the Teflon ring hanging by a number of metallic rings. A cylinder at whose base a tablet was attached was hung from this ring. The two sides of the balance were then balanced with a fixed weight on the right hand side. The mucus membrane was tied with mucosal side upward using a thread over a Teflon block. The block was then lowered into the jacketed beaker which was then filled with phosphate buffer such that buffer just reached the surface of the balance. The balance beam was raised by removing the fixed weight kept on the right side of the pan. This lowered the Teflon cylinder along with the tablet over the mucosa. The balance was kept in this position for a fixed time and then slowly increased on the right pan till the tablet separated from the mucus surface. The excess weight on right hand side gave the Bioadhesive strength of the tablet in grams. It was observed that assembly gave reproducible results and performed efficiently.

In vitro methods:

1. **Adhesion weight method:**

A system where suspension of an exchange resin particles flowed over the inner mucosal surface of a section of guinea pig intestine and the weight of adherent particles was determined. Although the method has limited value due to poor data reproducibility resulting from fairly rapid degradation and biological variation of the tissue, it was possible to determine the effect of particle size and charge on the adhesion after 5 minutes contact with the adverted intestine.

2. Flow channel method:

Mikos and Peppas developed this method which utilizes a thin channel made up of glass which is filled with 2% w/w aqueous solution of bovine submaxillary mucin, thermostated at $37_0{}^C$. Humid air at $37_0{}^C$ was passed through glass channel. A particle of Bioadhesive polymer was placed on the mucin gel, and its static and dynamic behaviour was monitored at frequent intervals using a camera, thereby calculating its adhesive property.

3. Fluorescent probe method:

In order to examine a large number of polymers for their Bioadhesive potential, the technique of labelling the lipid bilayer and memberane protein with the fluorescent probes namely pyrene and fluorescein isothiocynate, respectively, was used. Addition of polymers to this substrate

surface compressed the lipid bilayer or protein causing a change in fluorescence, as compared to control cells. By using the fluorescent probes, it was possible to compare charge type and density and backbone structure and their influence on polymer adhesion. Charged carboxylated polyanions were found to have a good potential for Bioadhesive drug delivery.

4. **Mechanical spectroscopic method:**

Mechanical spectroscopy was used to investigate the interaction between glycoprotein gel and polyacrylic acid, and the effect of pH and polymer chain length on this. Mortazavi et al, used a similar method to investigate the effect of carbopol 934 on the rheological behaviour of mucus gel. They also investigated the role of mucus glycoproteins and the effect of various factors such as ionic concentration, polymer molecular weight and its concentration, and the introduction of anionic, cationic and neutral polymers on the mucoadhesive mucus interface.

5. Thumb test:

It is simple test method used to quantify mucoadhesiveness. The difficulty of pulling the thumb from the adhesive as a function of pressure and contact time gives a measure of adhesiveness. It is most likely that any mucoadhesive system is adhesive to fingers, since most mucoadhesives are non-specific and not mucin specific and like mucin the skin has also many hydroxyl groups for interaction with Bioadhesive systems. Although the thumb test may not be conclusive, it provides useful information on mucoadhesive potential.

6. Colloidal Gold Staining:

This technique employed red colloidal gold particles, which were stabilized by the absorbed mucin molecules to form mucin gold conjugates. Upon interaction with mucin-gold conjugates, Bioadhesive hydrogel developed a red colour on the surface. Thus the interaction between them could easily be quantified, either by measurement of the intensity of the red colour on the hydrogel surface or by the measurement of the decrease in the concentration of the conjugates from the absorbance changes at wavelength.

7. Electronic conductance:

This method is used to test the semisolid mucoadhesive ointments. The adhesion of Orabase, carbopol, eudispert, guar gum and methylcellulose to artificial membranes in artificial saliva was studied by using a modified rotational viscometer capable of measuring electrical conductance. In the presence of adhesive the conductance was comparatively low, as the adhesive was removed, the value increased to final value, which corresponds to the conductance of saliva, which indicates the absence of adhesion

8. Folding endurance:

Folding endurance of patches was determined by repeatedly folding one patch at the same place till it broke or folded up to 200 times without breaking.

9. Drug content uniformity:

Drug content uniformity was determined by dissolving the patch by homogenization in 100 ml of an isotonic phosphate buffer (pH 7.4) for 8 h under occasional shaking. The 5 ml solution was taken and diluted with isotonic phosphate buffer pH 7.4 up to 20 ml, and the resulting solution was filtered through a 0.45 µm Whatman filter paper. The drug content was then determined after proper dilution at UVspectrophotometer. The experiments were carried out in triplicate.

10. Surface pH Determination:

The surface pH was determined by the method similar to that used by Bottenberg et al. 1991. A combined glass electrode was used for this purpose. The patches were allowed to swell by keeping them in contact with 1 ml of distilled water (pH 6.5±0.1) for 2 h at room temperature, and pH was noted down by bringing the electrode in contact with the surface of the patch, allowing it to equilibrate for 1 minute. The surface pH of the patches was determined in order to investigate the possibility of any side effects, in the oral cavity. As acidic or alkaline pH is bound to cause irritation to the buccal mucosa, hence attempt was made to keep the surface pH of the patch close to the neutral pH .

In vitro Swelling Studies of Buccoadhesive patch:

The degree of swelling of bioadhesive polymer is important factor affecting adhesion. Upon application of the bioadhesive material to a tissue a process of swelling may occur. The swelling rate of buccoadhesive patch was evaluated by placing the film in phosphate buffer solution pH 7.4 at 37^{o}C. Buccal patch was weighed, placed in a 2% agar gel plate and incubated at 37±10c. At regular one-hour time intervals (upto 3 h), the patch was removed from the petri dish and excess surface water was removed carefully using the filter paper. The swollen patch was then weighed again and the swelling index was calculated. Swelling index = W2- W1 W

In vitro release Studies:

In order to carry out *In-vitro* release studies dissolution test apparatus type II (USP) rotating paddle method was used. The studies were carried out for all formulation combination in triplicate, using 900 ml of isotonic phosphate buffer (pH 7.4) as the dissolution medium. The release was performed at 37^{0}C, at 50 rpm. To provide unidirectional release, one side of buccal patch was attached to a glass disk with the help of two sided adhesive tape then disk was put in the bottom of the dissolution vessel so that patch remained on the upper side of the patch remained on the upper side of the disk. An aliquot of 5ml sample was withdrawn at predetermined time intervals and similar volume was replaced with fresh phosphate buffer (pH 7.4) maintained at same temperature. Samples were then analyzed with the help of UV spectrophotometer.

***Ex vivo* mucoadhesion time:**

The selected batch was subjected to ex vivo mucoadhesion test. The disintegration medium was composed of 800 ml isotonic phosphate buffer pH 7.4 maintained at 37^{o}C. A segment of porcine cheek mucosa, 3 cm long, was glued to the surface of a glass slab, vertically attached to the apparatus. The mucoadhesive patch was hydrated from one surface using 15 and then the hydrated surface was brought into contact with the mucosal membrane.

The glass slab was vertically fixed to the apparatus and allowed to move up and down so that the patch was completely immersed in the buffer solution at the lowest point and was out at the highest point. The time necessary for complete erosion or detachment of the patch from the mucosal surface was recorded. The experiment was carried out in triplicate.

Permeation studies:

The *in vitro* study of venlafaxine permeation through the sheep buccal mucosa was performed using a Franz diffusion cell at 37 ± 0.2°C. Sheep buccal mucosa was obtained from a local slaughterhouse (used within 2 h of slaughter). Freshly obtained goat buccal mucosa was mounted between the donor and receptor compartments so that the smooth surface of the mucosa faced the donor compartment. The patch was placed on the mucosa and the compartments clamped together. The donor compartment was filled with 1 mL of isotonic phosphate buffer pH 7.4. The receptor compartment (15 mL capacity) was filled with isotonic phosphate buffer pH 7.4 and the hydrodynamics in the receptor compartment was maintained by stirring with a magnetic bead at 100 rpm. One mL sample was withdrawn at predetermined time intervals and analyzed for drug content at 224 nm.

Bioadhesion strength

The tensile strength required to detach the bioadhesive patch from the mucosal surface was applied as a measure of the bioadhesive performance. The apparatus was locally assembled. The device was mainly composed of a two-arm balance.

Marketed Products:

(www.authorstream.com 2012)

Oral bioadhesive formulation (tablets):

Corlan – hydrocartisone succinate
Bonjela – hypromellose
Taktarin – miconazole
Corsodyl – chlorohexidine

Buccal mucosa formulation:

Buccaten – nausea , vomiting, virtigo
Suscard - angina

Bucaal spray:

(www.medscape.com 2012)

Cannabis-derived buccal spray (sativex) for opoid resistance cancer
Ambien- zolpidem
Imitrex- sumatriptan

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MUCOADHESIVE DRUG DELIVERY SYSTEM-NASAL

Khan Halimunnisa, Hastak Vishakha

1. Introduction

Nasal drug delivery for systemic effects has been practiced since ancient times. In modern pharmaceutics, the nose had been considered primarily as a route for local drug delivery. A number of advances in pharmaceutical biotechnology in the last 2 decades resulted in possibilities for large scale productions of biopharmaceuticals especially proteins and peptides. The inability to administer these drugs by routes other than parenteral injection motivated scientists to explore other possibilities such as pulmonary and nasal administration. The initial enthusiasm was soon confronted with disappointing in vivo results showing poor bioavailability, typically in the order of <5–10% for large molecules. On the other hand, very good results were obtained with small organic molecules, which led to the successful development of a number of products currently on the market, list of products that is steadily increasing. Examination of the causes of failure led to the conclusion that the short residence time of the formulation within the nasal cavity coupled to the low permeability of the latter did play significant roles. Consequently, the attention shifted to the evaluation of mucoadhesive polymers, some of which would even demonstrate additional permeation-enhancing capabilities. The encouraging results and the desire to overcome some new challenges stimulated the development of new generations of polymers based on pH or thermal responsiveness or modified existing polymers having improved bioadhesive or permeation-enhancing properties. Even though a number of challenges are still to be overcome, especially with respect to toxicity, the potential of nasal drug delivery (NDD), including the ability to target drugs across the blood–brain barrier (BBB), are very high and continues to stimulate academic and industrial research groups so that we will keep witnessing increasing number of advanced nasal drug delivery products (Ugwoke M.I. 2005).

2. Biological and pharmaceutical considerations for nasal mucoadhesive drug delivery

2.1. Nasal anatomy and physiology relevant to nasal mucoadhesive drug administration

The nasal cavity is divided into two halves by the nasal septum and extends posteriorly to the nasopharynx, while the most anterior part of the nasal cavity, the nasal vestibule, opens to the face through the nostril.

The respiratory region, the nasal conchae or turbinates, which occupies the major part of the nasal cavity, possesses lateral walls dividing it into 3 sections. These folds provide the nasal cavity with a very high surface area compared to its small volume. The epithelial cells in the nasal vestibule are stratified, squamous and keratinized with sebaceous glands. Due to its nature, the nasal vestibule is very resistant to dehydration and can withstand noxious environmental substances and limits permeation of substances (Ugwoke M.I. 2005).

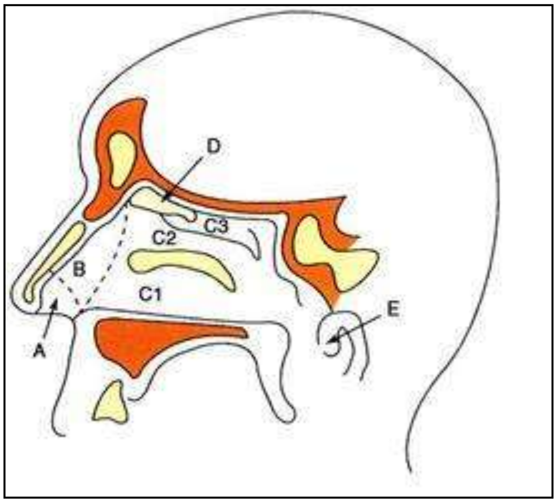

Fig.1. Section of human nasal cavity showing the nasal vestibule (A), atrium (B), respiratory region: inferior turbinate (C1), middle turbinate (C2) and the superior turbinate (C3), the olfactory region (D) and nasopharynx (E)

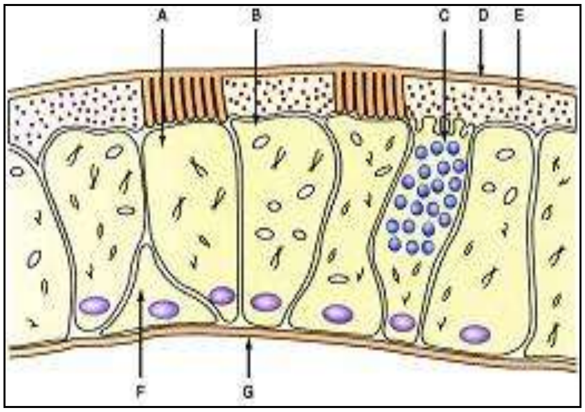

Fig.2. Cell types of the nasal epithelium showing ciliated cell (A), non-ciliated cell (B), goblet cells (C), gel mucus layer (D), sol layer (E), basal cell (F) and basement membrane (G)

2.2 Nasal absorption

The nasal absorption of drugs is considered mainly to take place in the respiratory region comprising the turbinates and part of the nasal septum. As is the case for all biological membranes, drugs can cross the nasal mucosal membrane using two different pathways; transcellularly – across the cell – and paracellularly – between the cells. Lipophilic drugs are transported transcellularly by an efficient concentration-dependent passive diffusion process, by receptor or carrier mediation and by vesicular transport mechanisms. Polar drugs are believed to pass through the epithelium via the gaps or pores between the cells (the tight junctions). Although, the tight junctions are dynamic structures that can open and close to a certain extent, the size of these channels is less than 10 Å. Hence, the paracellular route will be less efficient for large molecules and is dependent upon the molecular weight of the drug with a general molecular size cut-off of ~1000 Da. Lipophilic drugs, such as propanolol, progesterone, pentazocine and fentanyl, generally demonstrate rapid and efficient absorption when given nasally. For such drugs, it is possible to obtain pharmacokinetic profiles similar to those obtained after an intravenous injection with bioavailabilities for some drugs approaching 100%.

2.3. Nasal mucus secretion

The submucosal glands, which secrete nasal mucus, comprise both mucus cells and serous cells, producing a watery fluid. Seromucus glands in the Human nose have been estimated to 100,000. Mucus is also released from the goblet cells as mucus granules, which swell in the nasal fluids to contribute to the mucus layer.

Region	Structural features	Permeability
Nasal vestibule	Nasal hairs Epithelial cells are stratified, squamous and keratinized Sebaceous glands present	Least permeable because of keratinized cells
Atrium	Transepithelial region Stratified squamous cells present anteriorly and pseudostratified cells with microvilli present posteriorly Narrowest region of nasal cavity	Less permeable as it has small surface area and stratified cells present anteriorly
Respiratory region (inferior turbinate middle turbinate superior turbinate)	Pseudostratified ciliated columnar cells with microvilli (300 per cell), large surface area Receives maximum nasal secretions because of presence of seromucus glands, nasolacrimal duct and goblet cells Richly supplied with blood for heating and humidification of inspired air, presence of paranasal sinuses	Most permeable region because of large surface area and rich vasculature
Olfactory region	Specialized ciliated olfactory nerve cells for smell perception Receives ophthalmic and maxillary divisions of trigeminal nerve Direct access to cerebrospinal fluid	Direct access to cerebrospinal fluid
Naso-pharynx	Upper part contains ciliated cells and lower part contains squamous epithelium	Receives nasal cavity drainage

Table1. Structural features of different nasal anatomical regions and their relevance in drug permeability.

Mucus secretion is a complex mixture of many substances and consists of about 95% water, 2% mucin, 1% salts, and 1% of other proteins such as albumin, immunoglobulins, lysozyme and lactoferrin, and b1% lipids. The production of IgA by both the adenoid tissue and the nasal mucosa contributes significantly to the immune protection against inhaled bacteria and viruses. About 1.5–2 l of nasal mucus is produced daily. This mucus blanket, about 5 Am thick, consists of two layers, a lower sol layer and an upper gel layer. The viscosity of both layers affects ciliary beating and the efficiency of transporting the overlying mucus—the mucociliary clearance (MCC) (Ugwoke M.I. 2005).

The nasal mucus performs a number of physiological functions:

(1) It covers the mucosa, and physically and enzymatically protects it.
(2) The mucus has water-holding capacity.
(3) It exhibits surface electrical activity.
(4) It permits efficient heat transfer.
(5) It acts as adhesive and transports particulate matter towards the nasopharynx.

2.4. Mucociliary clearance

One of the functions of the upper respiratory tract is to prevent noxious substances (allergens, bacteria, viruses, toxins etc.) from reaching the lungs. When such materials adhere to, or dissolve in the mucus lining of the nasal cavity, they are transported towards the nasopharynx for eventual discharge into the GIT. Clearance of this mucus and the adsorbed/dissolved substances into the GIT is called the MCC. It consists of a coordinated interaction between the overlying mucus layer and the methachronal wavelike movement of the underlying cilia. Optimum physico-chemical properties of the mucus and movement of the cilia are required for effective and efficient MCC. There are approximately 300 cilia per cell, each cilia is 5–10 Am long and 0.1–0.3 Am wide and beats as a frequency of about 20 Hz.

The MCC can be influenced by environmental and pathological conditions. Factors that can increase ciliary beat frequency (CBF) and mucus production, or decrease mucus viscosity will all lead to increase in MCC. Environmental conditions like temperature (below or above 23˚C), inhalation of sulfur dioxide and cigarette smoke decrease MCC. Pathological conditions like Kartagener’s syndrome, Sjorgens syndrome, asthma, nasal polyposis, defective septum, rhinitis, allergy, common cold and sinusitis alter MCC due to their effects on CBF or mucus rheology (Ugwoke M.I. 2005).

2.5. Mucus–drug interaction: relevance to nasal mucoadhesive drug delivery

It is an important consideration to understand how the mucus and drugs interact with each other, and even more importantly how such interactions could influence drug absorption and activity. Such consideration includes the diffusion coefficient of the drug through the mucus in disease states. The drug’s molecular size, the mucus mesh size and the interaction between the mucus gel and the drug influence the diffusion of drugs through the mucus. Like in all cases of diffusion, small and neutral molecules diffuse faster and to a greater extent through a charged matrix compared to charged and large molecules. Some large or charged molecules have been observed to diffuse readily through the mucus. Additionally, particulate materials, e.g., microspheres and liposomes bearing drugs, have been observed to diffuse through the mucus and reach the epithelium intact.

A full understanding of the underlying factors that influence diffusion through mucus is presently lacking. Particulate transport through the mucus is rather complex and is controlled by the mucus layer as well as size, charge and surface wettability of the particles. Studies on particulate transport through the mucus all demonstrated that diffusion is size dependent and decreases quickly with increasing size. A study of all the possible and complex interactions can help not only to avoid problems resulting from such interactions (low drug absorption) but also to exploit the situation for the enhancement of drug transports through the mucus. Alteration of the formulation components by changing pH, ionic strength, polymer, surface charge of nanoparticles and microparticles, etc., can sufficiently alter the gel structure to the extent that increased absorption can take place (Ugwoke M.I. 2005).

3. Mucoadhesion

3.1 Mechanism of mucoadhesion (Chaturvedi M. 2011)

The process of mucoadhesion following nasal administration relates to the interaction between the mucoadhesive polymer and the mucus secreted by the sub-mucosal glands. The sequential events that occur during the mucoadhesion include the proper wetting and swelling of the polymer, and intimate contact between the polymer and the nasal mucosa. Then, the swelled mucoadhesive polymer penetrates into the tissue crevices followed by the interpenetration between the polymer chains and protein chains of the mucus. To obtain sufficient absorption of drugs, first, the formulation should spread well on the nasal mucosa. Therefore, the spreadability is very important for the liquid mucoadhesive formulation, so do the flowability and wettability for the solid mucoadhesive formulation.

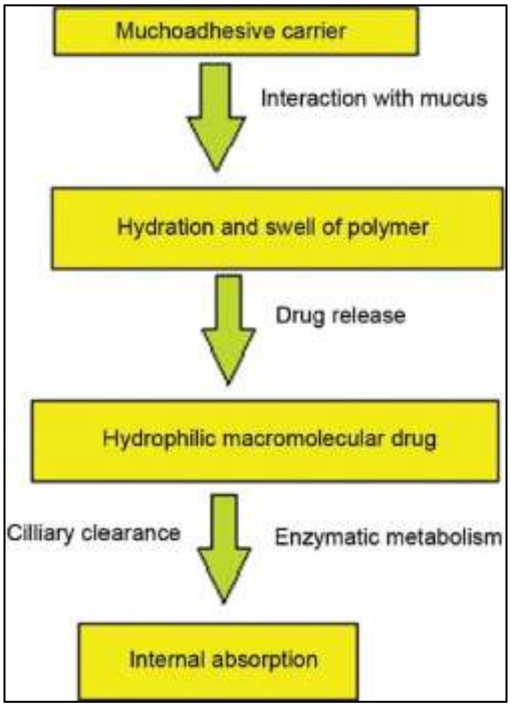

Fig.3 The process of mucoadhesion (Chaturvedi M. 2011)

Hydration of the polymer (swelling) plays a very important role in mucoadhesion, through which the polymer chains are liberated and interact with the biological tissue. During hydration, there is a dissociation of hydrogen bonds of the polymer chains. When the polymer–water interaction becomes greater than the polymer–polymer interaction, adequate free polymer chains will be available for interaction between the polymer and biological tissue.

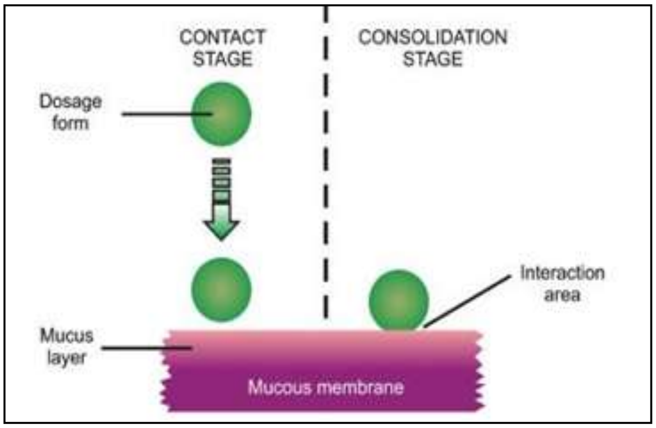

Fig.4 The two steps of mucoadhesion process

There is a critical degree of hydration required for optimum mucoadhesion. The incomplete hydration due to the lack of the water leads to incomplete liberation of the polymer chains. On the other hand, an excessive amount of water will weaken the mucoadhesive bonds by over diluting the polymer solution. The polymer chains penetrating into the tissue crevices can hold back the ciliary movement, which will increase the retention time of the drugs in the nasal cavity.

Furthermore, the existence of the mucoadhesive carrier also reduces the contact between the drugs and the enzymes existing in the mucosa. These both can enhance the intranasal absorption of hydrophilic drugs. Apart from these, the dehydration of the epithelial cells after hydration may also temporarily open the tight junctions between the epithelial cells and improve the paracellular absorption of macromolecular drugs. This function of the mucoadhesive polymer is very important for the enhancement of the intranasal absorption of macromolecules weighting above 1000 Da. Mucoadhesion can slow down the mucociliary clearance, but with time, mucus production will lead to the inordinate swelling of the mucoadhesive polymer and the reduction of the mucoadhesion bond strength, allowing a recovery of normal mucociliary movement rate and the clearance of the polymer from the mucosa.

Disease conditions can affect mucoadhesion due to their influence on either mucus production or ciliary movement, and then may result in undesired drug release. Thus, a good understanding of the nature of mucus in these diseases is imperative in designing a good nasal drug delivery system. Mucoadhesive capabilities of polymers should be studied under such disease conditions during the product development.

3.2 Mucoadhesion as a strategy to improve systemic drug delivery via the nasal route

A very rapid rate of absorption can be achieved following nasal application of some drugs. Frequently (depending on the physicochemical characteristics of the drug) this is accompanied by high bioavailability.

This recognition of the potential of NDD has led to an explosion of research (both fundamental and applied) in this field in the last 2 decades. The list of nasal drug products in the market or at various stages of preclinical and clinical development is ever increasing. These developments are supported by the recognition of the advantages the nose presents for drug delivery purposes. These include:

Systemic nasal absorption of drug is a new attractive alternative to parenteral drug delivery system, as it offers the following advantages (Ugwoke M.I. 2005):

1. A large surface area is available for drug deposition and absorption. The effective absorptive surface area of the nasal epithelium is even higher as a result of the presence of microvilli.
2. The nasal epithelium is thin, porous (especially when compared to other epithelial surfaces) and highly vascularized. This ensures high degree of absorption and rapid transport of absorbed substances into the systemic circulation for initiation of therapeutic action.
3. The rate and extent of absorption as well as plasma concentration vs time profiles are comparable with I.V. administration.
4. A porous endothelial basement membrane that poses no restriction to transporting the drug directly into systemic circulation, e.g. Amastatin, Puromycin, Nifedipine, etc.
5. Unlike the skin, nasal mucosa is not constructed from the keratinized stratum corneum. The subepithelial layer of the nasal mucosa with numerous microvilli is highly vascularized, with large and fenestrated capillaries facilitating rapid absorption.
6. Avoidance of first pass elimination, gut wall metabolism, and destruction in gastrointestinal tract (Zakia N.M. 2007)
7. In some cases, drugs can be absorbed directly into the CNS after nasal administration bypassing the tight blood–brain barrier.
8. The enzymatic activity of the nasal epithelium is lower than that of the GIT or liver and higher bioavailability of drugs especially proteins and peptides can be achieved. In addition, enzyme inhibitors are more effective following nasal than oral application because of a higher degree of dilution in the latter than in the former.
9. Realization of pulsatile delivery of some drugs like human growth hormone, insulin, etc., is higher with NDD.
10. The nose is amenable to self-medication that not only lowers the cost of therapy but improves patient compliance compared to parenteral routes.
11. Various nasal drug delivery systems are available for user-friendly noninvasive painless application.
12. The risk of over-dosage is low and nasal lavage can be used to remove unabsorbed excess drug.
13. Reformulation of existing drugs as NDD products offers companies the possibility to extend the life cycle of their products.

Limitation (Chaturvedi M. 2011):

1. Only a limited amount of the formulation can be administered intranasally. Application of large quantities will disturb the normal functioning of the nose (olfaction and humidification of inspired air).
2. It could also lead to irreproducibility of the dosing regimen as a result of drainage of the solution or expulsion of the dose due to sneezing.
3. The high porosity of the nasal epithelium is still not sufficient for absorption of all compounds especially hydrophilic ones and large molecules like proteins.
4. In addition, the nasal mucosa is enzymatically active albeit to a lesser extent compared with the GIT.
5. Low bioavailability results from enzymatic degradation and metabolism at mucosal site and low residence time.

6. Stringent requirement is placed on the administration device for reproducibility of deposition.

7. Drug absorption and permeability of the different regions of the nasal cavity is quite different. Also deposition posteriorly will result in faster clearance by the MCC (Bertram U. 2006)

8. There is risk of local side effects and irreversible damage of the cilia on the nasal mucosa, both from the drug substances and from the constituents added to the dosage forms.

9. Certain compounds when used as absorption enhancers may disrupt and even dissolve the nasal membrane in high concentration.

10. Nasal atrophic rhinitis and severe vasomotor rhinitis can reduce the capacity of nasal absorption, e.g., Caerulein.

11. There could be mechanical loss of the dosage form into the other parts of the respiratory tract like lungs.

12. Untoward immunogenic effects might arise with the route.

13. Major factors limiting the bioavailability of nasally administered protein drugs include (Jain A.K. 2008)

(a) Poor permeability across lipophilic membranes due to hydrophilicity of these agents,

(b) Poor permeability via the paracellular route due to large size, and/or

(c) The mucociliary clearance mechanism that rapidly removes the non-mucoadhesive formulations from the absorption site.

3.3 Factors affecting Mucoadhesion (Tangri P. 2011):

Polymer based factors

- molecular weight of the polymer
- concentration of polymer used
- flexibility of polymer chains
- swelling factor
- stereochemistry of polymer

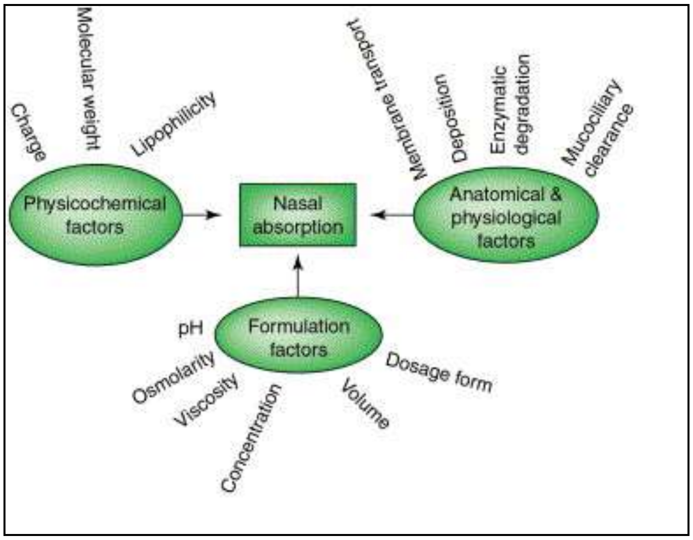

Fig.5 Various factors affecting mucoadhesion (Illeum L., 2002)

Physical factors

- pH at polymer substrate interface
- applied strength
- contact time

Physiological factors

- mucin turn over rate
- diseased state

3.4 Nasal absorption enhancement

It is possible to greatly improve the nasal absorption of polar drugs by administering them in combination with an absorption enhancer that promotes the transport of the drug across the nasal membrane. Furthermore, a nasal drug-delivery system that combines an absorption enhancing activity with a bioadhesive effect, which increases the residence time of the formulation in the nasal cavity, has been shown to be even more effective for improving the nasal absorption of polar drugs. However, caution is required when interpreting results obtained in animal models for the transport promoting effect of nasal absorption enhancers. Especially with the rat model (*in situ*, anaesthetized and otherwise), the effect obtained by enhancers can be grossly overestimated. However, other animal models, can give misleading results, most probably a result of the different architecture and morphology of the nasal cavity and also the use of anaesthetics or sedating procedures that can affect the mucociliary clearance mechanism. Generally, for enhancer systems there is a direct correlation between the bioavailability obtained and damage caused to the membrane. For other enhancers, especially those that work by transiently opening the tight junctions between the cells e.g. chitosan, selected cyclodextrins and phospholipids, the absorption enhancing effect can greatly outweigh any modifications caused to the mucosa.

4. Mucoadhesive nasal drug delivery

4.1 Targets for Nasal delivery (Illeum L. 2002)

Local delivery

- Nasal allergy
- Nasal congestion
- Nasal infection

Systemic delivery

- Crisis treatments – rapid onset is needed
- Long term treatment – daily administration
- Peptides and proteins – difficult to administer

Vaccine delivery

- Antigens (whole cells, split cells, surface antigens)
- DNA vaccines

Access to CNS

- To reach local receptors
- To circumvent the blood-brain barrier

4.2 Main reasons for exploiting the nasal route for vaccine delivery:

- The nasal mucosa is the first site of contact with inhaled antigens
- The nasal passages are rich in lymphoid tissue (Nasal Associated Lymphoid Tissue-NALT or Waldeyer's ring in humans)
    - Adenoid or nasopharyngeal tonsils
    - Bilateral lymphoid bands
    - Bilateral tubal and facial or palatine tonsils
    - Bilateral lingual tonsils
- Creation of both mucosal (IgA) and systemic (IgG) immune responses
- Low cost, patient friendly, non-injectable, safe.

4.3 Nasal mucoadhesive delivery of pharmaceutical compounds (Ugwoke M.I. 2005)

4.3.1. Small organic molecules

Due to the rapid therapeutic action that can be achieved, medications used in emergency medical situations make ideal candidates for nasal drug delivery. One such drug, apomorphine is the drug of choice for treatment of on/off-syndrome in patients suffering from Parkinson's disease. Aqueous solution of the compound is reasonably well absorbed following nasal administration with a relative bioavailability of 45%. It is further demonstrated that even higher relative bioavailability (98%) is possible, if drainage of the solution through the nasopharynx is limited and rapid oxidation of the aqueous solution, with a degradation half-life in plasma at 37ºC of 39 min. This was achieved by administering the drug in powdered form. It is demonstrated in several studies that the pharmacokinetic profiles of apomorphine after nasal administration may be improved following incorporation of the compound into mucoadhesive polymers like polyacrylic acid, carbopol and carboxymethylcellulose. These studies showed that nasal relative bioavailability of apomorphine powders was higher than that of solutions and was equivalent to subcutaneous injections.

Although the nasal mucoadhesive formulations pharmacokinetic profiles than nasal solutions, the study showed that physicochemical characteristics of a drug molecule and its release from the polymer matrix could affect the rate and extent of drug absorption. Therefore, incorporation of a rapid release excipient (e.g., lactose) may be of relevance as demonstrated for apomorphine formulated with Carbopol 974P.

In addition to apomorphine, other small molecular weight compounds including budesonide, caffeine, ketorolac, metoprolol, midazolam, morphine-6-glucoronate, nicotine, oxyprenolol, oxymetazoline and pentazocine have been characterized for nasal administration with mucoadhesives.

The efficacy of drugs (e.g., steroids, bronchodilators) targeted to the respiratory mucosa for diseases such as rhinitis and asthma generally depends on duration of action, and thus on drug residence time in the mucosa. For fluticasone propionate, retention may be achieved exclusively by lipophilicity, whereas for budesonide an additional possibility may be provided by its ability to form fatty acid esters in the airway mucosa that release the active drug or the use of mucoadhesives. Polymers containing carboxylic acid groups adhere strongly to epithelial mucosa due to hydrogen bond interactions in environments where the pH is less than 5, and the carboxylic acid does not dissociate. As with budesonide, mucoadhesive formulation of xylometazoline was shown to exhibit significantly longer clinical effect (manometric/subjective scores) than non-mucoadhesive product. This was ascribed to the adhesiveness of the formulation, which allowed the adrenergic agonist to stay longer at the site of application resulting in prolonged and more pronounced effect. Morphine when given orally, the bioavailability is approximately 20–32%. It was demonstrated that chitosan-based mucoadhesive formulation of the compound resulted in rapid absorption of the compound (Tmax of 15 min) with a bioavailability of about 60%. The plasma profiles after nasal administration with chitosan were better than simple liquid formulations, but similar to those obtained after intravenous doses.

4.3.2. Antibiotics

Many antibiotics are still exclusively administered via parenteral routes. For those administered orally, some exhibit erratic and incomplete absorption. Recently a few studies have examined the potential of the nasal route for systemic delivery of antibiotics using mucoadhesive polymers. It is showed that hyaluronic acid and chitosan may be employed for nasal administration of antibiotics to obtain a high bioavailability and prolonged release. In another study, Canan et al. suggested, based on particle size, production yield, encapsulation efficiency, shape and surface properties, drug–polymer interaction, mucoadhesive property and in vitro drug release that hydroxypropyl methylcellulose is a good polymer for systemic delivery of gentamicin via the nasal route. Successful nasal delivery of other antibiotics such as vancomycin and tobramycin with chitosan has been reported as well. It was shown that the presence of chitosan salts slow down the release of vancomycin hydrochloride at pH 5.5 and pH 7.4, thus guaranteeing a sustained release at acidic and alkaline pH of drug in the nasal cavity. Studies involving nasal delivery of ciprofloxacin hydrochloride using hydroxypropyl methylcellulose (HPMC), hydroxyethyl cellulose (HEC), and methylcellulose (MC) showed that the bioavailability of ciprofloxacin gel formulation prepared with HPMC was almost identical to that of the oral route, but the bioavailabilities for HEC and MC were lower than oral preparations.

Although it is very interesting to deliver antibiotics through the nasal route, the use of mucoadhesives raises important safety concerns due to the prolonged drug–polymer residence time in the nasal mucosa, which may lead to superinfection in the respiratory tract. The increased contact time may upset the nasal normal microbial flora when broadspectrum antibiotics are used. This may lead to superinfection as documented for the gastrointestinal mucosa. This should be considered when selecting antibiotics to the delivered nasally using mucoadhesives.

Critical factors in designing nasal delivery systems

Physicochemical factors	Formulation factors	Physiological factors
Chemical form Polymorphism Molecular weight Particle size Stability and dissolution rate	pH and buffering capacity Osmolarity Gel forming carriers/ viscofying carriers Solubilisers Preservatives Antioxidants Drug concentration Drug volume Uptake promoters	Effect of disposition Nasal blood flow Mucociliary clearance Enzymatic degradation Pathological condition

Table. 2 Various formulation barriers

4.3.3. Macromolecules

4.3.3.1. Vaccines and DNA

Pathogenic infections in such disease states as influenza, pertussis, meningitis, measles, etc., occur primarily as a result of the pathogens gaining access to the body via mucosal contact. Consequently these diseases are ideal candidates where nasal vaccination can be applied since neutralizing antibodies and specific cellular responses at these sites of pathogen entry can take place.

It has been demonstrated that both systemic and mucosal immunity can be induced following nasal vaccination in humans. The major attraction of adjuvants like chitosan includes its low toxicity, susceptibility to biodegradation, mucoadhesive properties and drug penetration enhancement capacity across mucosal barriers. The type of chitosan formulation used (solution or powder) for the nasal administration of the antigen may affect the efficiency of the immune response and possibly the permeation of the antigens across the nasal mucosa. The ability of low molecular weight chitosans to trigger long-lasting humoral and mucosal immune responses is also an important attribute. Chitosan has been reported to significantly enhance immune response of nasally administered vaccines (e.g., influenza, pertussis and diphtheria vaccines) via induction of serum IgG responses similar to secretory IgA levels and superior to what was induced by a parenteral administration of the vaccine.

It was shown by Park et al. that the use of in situ gelling and mucoadhesive polymer vehicles (poloxamers, polycarbophil or polyethylene oxide) could effectively and safely improve the nasal retention and absorption of plasmid DNA. The rate and extent of nasal absorption could be controlled by choice of mucoadhesive polymers and their contents.

4.3.3.2 Proteins

Nasal delivery of peptides and proteins

- Nasal salmon calcitonin (Novartis)
- Nasal desmopressin (Ferring and partners)
- Nasal buserelin (Aventis)
- Nasal nafarelin(Searle)
- Nasal PTH, nasal leuprolide, nasal insulin, nasal interferon, etc.

Due to high molecular weight of proteins, rapid mucociliary clearance and enzymatic degradation, low absorption of these compounds occurs following nasal administration. Some mucoadhesive polymers have been suggested to extend residence time and improve uptake of large molecules across the nasal mucosa.

Garcia et al. showed that incorporation of cyanocobalamin into microcrystalline cellulose, dextran microspheres, and crospovidone resulted in significant improvement in bioavailability of cyanocobalamin relative to simple nasal solutions in rabbits.

Insulin is one of the most widely studied proteins with respect to nasal delivery using mucoadhesives. The strategies adopted to optimize the use of mucoadhesives for nasal delivery of insulin include preparing the formulations as micro- or nanospheres, incorporation of absorption enhancers and combination of different polymers. Dyer et al. showed that for chitosan, the most effective formulation for nasal insulin absorption is a chitosan powder delivery system, which was found to be better than chitosan nanoparticles and chitosan solution formulations, respectively.

Using different proportions of various mucoadhesives, Callens and Remon reported that following nasal administration of insulin formulated with drumdried waxy maize starch (DDWM) or maltodextrins and Carbopol 974P, the highest absolute bioavailability attained was 14.4% for a mixture consisting of DDWM/Carbopol 974P 90/10. The study identified drug loading and lyophilization as the critical parameters requiring optimization prior to applying these polymers for nasal delivery of insulin. Differences in nasal bioavailability between the different carriers could be explained by differences in GV (storage modulus, elasticity) and GU (loss modulus, viscosity) values.

Using hypoglycemia as therapeutic index, Wang et al. showed that intranasal administration of insulin with aminated gelatin significantly increased hypoglycemia compared with intranasal administration of insulin in phosphate buffered saline. This indicated that aminated gelatin effectively enhanced the nasal absorption of insulin. In a related study, it was shown that blood glucose lowering effect of glucagon nasal powder formulated with microcrystalline cellulose was preferred over liquid formulations because of higher stability, reduction of irritation and better dispersion.

4.4 Nasal mucoadhesive pharmaceuticals

The nasal route is important for drugs that are used in crisis treatments, such as for pain, and for centrally acting drugs where the putative pathway from nose to brain might provide a faster and more specific therapeutic effect. The world market has seen an increasing number of systemically acting drugs being marketed as nasal formulations. For example, Sumatriptan (GlaxoSmithKline), Zolmitriptan (AstraZeneca), Ergotamine (Novartis), Butorphanol (Bristol-MyersSquibb), all with the indication for treatment of migraine, where a rapid onset of action is beneficial; Estradiol (Servier), where an improved bioavailability as compared to oral delivery has been achieved; and Desmopressin (Ferring), Buserelin (Aventis) and Calcitonin (Novartis), all peptides normally only administered by injection because of low membrane permeability and susceptibility to degradation by enzymes in the gastrointestinal tract.

A range of companies specializing in the development of innovative nasal delivery systems and formulation problems has come to the fore: Nastech, Britannia Pharmaceuticals, Intranasal Technologies, Bentley Pharmaceuticals and West Pharmaceutical Services are actively developing novel nasal formulations for conventional generic drugs (e.g. apomorphine, triptans, morphine, midazolam, fentanyl, non-steroid anti-inflammatory drugs), as well as for peptides and proteins (e.g. leuprolide, parathyroid hormone, insulin, interferon) in situations where the nasal route would be beneficial for the therapeutic efficacy of the drug.

Furthermore, the use of the nasal cavity for vaccination, especially against respiratory infections, is being pursued by vaccine companies. This is because it is possible to obtain, by the nasal route, not only a systemic immune response, but also a local mucosal immune response that should provide a much higher level of protection against these diseases. The first nasal influenza vaccine from Berna Biotech based on an antigen–adjuvant system, reached the European market in 2001 and a second influenza vaccine from Aviron (cold adapted virus system) was launched in 2003.

5. Mucoadhesive polymers used in nasal drug delivery (Chaturvedi M. 2011)

Hydrophillic polymers

Contains carboxylic group and possess excellent mucoadhesive properties. These are,

- PVP(Poly vinyl pyrrolidine)
- MC(Methyl cellulose)
- SCMC(Sodium carboxy metyhyl cellulose)
- HPC(Hydroxyl propyl cellulose)

Hydrogels

These swell when in contact with water and adhere to the mucus membrane. These are further classified according to their charge

- Anionic polymers- carbopol, polyacrylates
- Cationic polymers- chitosan
- Neural/ non ionic polymers- eudragit analogues

5.1 Cellulose Derivatives

Several cellulose derivatives have proved to be effective on enhancing the intranasal absorption of drugs, including soluble cellulose derivatives such as hydroxypropyl methylcellulose, hydroxypropyl cellulose (HPC), methylcellulose (MC), and carboxymethyl cellulose (CMC), and insoluble cellulose derivatives such as ethylcellulose and microcrystalline cellulose (MCC).

Cellulose derivatives can markedly prolong the residence time of drugs in the nasal cavity due to their desirable mucoadhesive property. Additionally, due to their high viscosity following hydration in the nasal cavity, the celluloses can sustain the release of drugs. For these reasons, using celluloses as absorption enhancer can lead to improved intranasal absorption and increased bioavailability. Many references show that the celluloses are effective on increasing the intranasal bioavailability of small hydrophobic as well as hydrophilic macromolecular drugs.

Mucoadhesive polymer	Drug	Dosage form
CMC	Apomorphine	Powder
MCC	Ketorolac acid	Spray
MCC/HPC	Leapralide	Powder
HPC	Dopamine	Liquid
HPC	Metaclopramide	Gel

Table.3 Nasal drug delivery systems with cellulose derivatives

5.2 Polyacrylates

Polyacrylates have been used frequently in many drug administration routes, due to their excellent mucoadhesive and gel-forming capability. Among the pharmaceutical polyacrylates, carbomers, and polycarbophil, which differ in the cross-linking condition and viscosity, are widely used in the nasal mucoadhesive drug delivery systems. Polyacrylates, capable of attaching to mucosal surfaces, can offer the prospects of prolonging the residence time of drugs at the sites of drug absorption, and ensure intimate contact between the formulation and membrane surface. Sustained release of drugs can also be obtained by using polyacrylates in nasal formulation, which result in a more stable blood concentration-time curve. Besides the mucoadhesion capability, polyacrylates may also temporarily open the tight junctions between the epithelial cells during the swelling progress in the nasal cavity and improve the paracellular absorption of drugs.

Mucoadhesive polymer	Drug	Dosage form
Carbopol 971P	Apomorphine	Powder
Carbopol 934P	Levonorgestrol	Liquid
Carbopol 981P	Metaclopramide	Powder
Carbopol 934/HPC	Metaclopramide	Powder

Table.4 Nasal drug delivery systems with polyacrylates

5.3 Starch

The starch is one of the most widely used mucoadhesive carrier for nasal drug delivery, which has been reported to be effective on improving the absorption of both small hydrophobic drugs and hydrophilic macromolecular drugs. Maize starch is the most preferred class for pharmaceutical purpose, among which the drum-dried waxy maize starch, due to its better bioadhesive property, has been considered as the best one compared with starch processed through other methods. Starch can be used as nasal drug carriers in the form of powder microspheres and nanoparticles among which the degradable starch microspheres (DSM), also known as SpherexR, is the most widely used and also the first example of mucoadhesive microparticulate nasal delivery system.

5.4 Chitosan

Chitosan and its derivatives have been shown to be active in enhancing the intranasal drug absorption due to their excellent mucoadhesive properties. It was also confirmed that coating micro- and nanoparticulates with chitosan could improve drug adsorption to mucosal surfaces. It is reported that chitosan microspheres and solutions resulted in three- and eight-fold longer clearance half-lives compared with sodium pertechnetate solution in sheep nasal cavity, respectively. In addition, many studies have proved that chitosan and its derivatives could transiently open the tight junctions between the cells and lead to the paracellular transport of drugs.

Chemical and biological properties of chitosan, such as mucoadhesion and ability in enhancing nasal absorption, are determined by the types of derivatives, degree of deacetylation, and molecular weight, because chitosan is only soluble in acidic environment in which the amino groups at the C-2 position are protonated. At neutral pH, most chitosan molecules will lose their charge and precipitate from solution. To improve the poor water solubility of chitosan, some derivatives were synthesized, such as trimethyl chitosan. Due to the positive charge of chitosan in a weak acidic environment, it can also be applied to deliver the negatively charged DNA through nasal mucosa and protect them from nuclease degradation.

Compared with viral vectors, this alternative vector markedly reduced the safety risks that meanwhile result in high transfectability. Recently, many studies show that nasal immunization with chitosan plus inactive vaccine is a potentially effective, easily administered form of vaccination. It is reported that chitosan solutions are able to enhance both the mucosal and systemic immune responses against influenza virus vaccines.

5.5 Polymer properties affecting mucoadhesion (Andrews G.P., 2009)

Functional group contribution

The attachment and bonding of bioadhesive polymers to biological substrates occurs mainly through interpenetration followed by secondary non-covalent bonding between substrates. Given that secondary bonding mainly arises due to hydrogen bond formation, it is well accepted that mucoadhesive polymers possessing hydrophilic functional groups such as, carboxyl (COOH), hydroxyl (OH), amide (NH2) and sulphate groups (SO4H) may be more favourable in formulating targeted drug delivery platforms. Typically, physical entanglements and secondary interactions (hydrogen bonds) contribute to the formation of a strengthened network; therefore polymers that exhibit a high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins. Mucoadhesive polymers are generally hydrophilic networks that contain numerous polar functional groups. Such functionalised polymers interact with the mucus not only through physical entanglements but also through secondary chemical bonds, thus resulting in the formation of weakly cross-linked networks. The key sites for mucoadhesive interactions appear to be on the carbohydrate residues, via electrostatic interaction or through hydrophobic bonding of fucose clusters.

Degree of hydration

Another important factor affecting the mucoadhesive strength of polymeric components is the degree of hydration. Many polymers exhibit adhesive properties under conditions where the amount of water is limited. However in such a situation, adhesion is thought to be a result of a combination of capillary attraction and osmotic forces between the dry polymer and the wet mucosal surface which act to dehydrate and strengthen the mucus layer. While hydration is essential for the relaxation and interpenetration of polymer chains, excess hydration could lead to decreased mucoadhesion and/or retention due to the formation of slippery mucilage. In this situation cross-linked polymers that only permit a certain degree of hydration may be advantageous for providing a prolonged mucoadhesive effect.

Polymer molecular weight, chain length, conformation, and degree of cross-linking

It is well accepted that structural polymeric components significantly influence the extent of diffusion, entanglement and hence mucoadhesion. A large molecular weight is essential for entanglement; however, excessively long polymer chains lose their ability to diffuse and interpenetrate mucosal surfaces.
The degree of cross-linking within a polymer system significantly influences chain mobility and resistance to dissolution. Cross-linked hydrophilic polymers swell in the presence of water allowing them to retain their structure, whereas similar high molecular weight linear hydrophilic polymers are swellable and readily dispersible. In mucoadhesive terms swelling is favourable as it not only allows greater control of drug release, but also additionally the swelling process increases the surface area for polymer/mucus interpenetration. As cross-link density increases, chain mobility decreases and hence the effective chain length, which can penetrate into the mucus layer decreases, reducing mucoadhesive strength. Chain flexibility is critical for interpenetration and entanglement with the mucus gel. Increased chain mobility leads to increased inter-diffusion and interpenetration of the polymer within the mucus network.

pH and charge

The charge density of macromolecules is an important factor for bioadhesion with polyanions preferred to polycations when considering both toxicity and bioadhesion. Macromolecular charge is affected by the pH of the physiological environment due to the dissociation of functional groups. There is the greatest potential for polymer mucus hydrogen bonding with undissociated anionic pendant functional groups. In relation to carboxylated polymers, pH values below the respective pKa value would then be more favourable.
While it is recognised that mucoadhesion processes are optimised in low pH environments, mucoadhesion may not be completely lost at higher

pH values. At higher pH levels, repulsion of like functional groups changes the spatial conformation from a coiled state into a ‘‘rod-like” structure making them more readily available for inter-diffusion and interpenetration. Interestingly, above the pKa of mucin a net negative charge may result in the repulsion of anionic species such as observed in ionised polyacrylic acid systems. At such elevated pH values, positively charged polymers, such as chitosan, may form polyelectrolyte complexes with negatively charged mucins and exhibit strong mucoadhesion.

Mucoadhesive polymers may be divided into three main groups in terms of overall charge, i.e., anionic, cationic and non-ionic systems. Anionic polymer systems such as the polyacrylic acids make up the bulk of pharmaceutically employed mucoadhesive polymers.

Polymer concentration

Polymer concentration has also been shown to significantly influence the strength of mucoadhesion. Optimal polymer concentration is dependent on physical state of the delivery system, with observational differences between semisolid and solid-state platforms.

In the semisolid state, an optimum concentration exists for each polymer beyond which reduced adhesion occurs because a lower number of polymer chains are available for interpenetration with mucus.

Environmental and physiological factors

The mucus turnover at the applied surface/site is variable. Mucus gel layer viscosity can vary throughout the body, with variability increasing in certain disease states. Low mucus viscosity results in a weak, easily detachable polymer/mucus bond, making targeted drug delivery extremely difficult. In contrast an extremely viscous mucus layer, such as those thickened due to white blood cell DNA, dead cells and inflammatory mediators limits the degree of interpenetration and also increases the diffusion pathway through which the active agent must pass.

Furthermore, the ionic strength of the surrounding medium may also have a significant role in defining the mucoadhesive bond force. In general mucoadhesion strength is decreased in the presence of ions due to shielding of functional sites that are pertinent for adhesion processes and importantly, gel network expansion.

6. Various dosage forms given by nasal route (Chaturvedi M. 2011):

Suspensions

Suspensions for nasal administration are prepared by suspending the micronized drug in a liquid diluent or carrier suitable for application to the nasal mucosa. The preparation of suspension form gave a better insulin uptake and blood glucose reduction compared with that from the solution.

Powders

Powder dosage forms of drugs for nasal administration offer several advantages over liquid formulations. In the powder form, the chemical stability of the drug is increased, a preservative in the formulation is not required, and it is possible to administer larger doses of drugs. Powder form is suitable for number of non-peptide drugs and is well suited for peptide drugs.

Polymer-based powder formulations show no adhesion until their absorption of mucus occurs on the nasal mucosa surface. This allows easy application to the nasal cavity by metered dose insufflation even if the polymer is highly mucoadhesive. In addition, liquid preparations are more easily cleared to the nasopharynx and oropharynx from where they enter the posterior part of the tongue. Therefore, administration of nasal powders may increase patient compliance, especially if the smell and taste of the delivered drug is unacceptable. After getting in contact with the nasal mucosa, polymer-based powders are believed to form a viscous gel following absorbing water from the nasal mucus. Then, the free polymer chains penetrating into the tissue crevices can hold back the ciliary movement, which will increase the retention time of the drugs in the nasal cavity. Dry powder formulations can also avoid the utilization of preservatives and freeze storage, because they do not support microbial growth and are more stable than solution. For these reasons, the dried powder is the most commonly studied formulation for the nasal drug delivery, including small hydrophobic drugs, peptide drugs, and vaccine. Dry powder nasal influenza vaccine formulation by using spray-freeze-drying method; the results indicated that the powders were amorphous and more stable with respect to liquid formulations.

Nasal Particulate Drug Delivery System

Nasal particulate systems using mucoadhesive polymers as carriers include microparticle/sphere and nanoparticle. Particulate drug carrier systems administered through nasal mucosa may protect the drug from enzymatic degradation, increase the drug dissolution rate, intensify the contact of the formulation with the mucosa, enhance the uptake by the epithelium, and act as a controlled release system resulting in prolonged blood concentrations. Among the polymers widely used as nasal drug particulate carrier, the positively charged polymers such as chitosan and aminated gelatin are most attractive because of their hydrogel nature which leads to opening of the tight junctions and their intimate contact with the negatively charged mucosa membrane. It has been believed that nanoparticles possess superiority over microspheres as nasal drug carrier because their larger surface area results in more intimate contact with the mucosa, which leads to higher local concentration gradient. Moreover, nanoparticles cross the mucosal epithelium better than microspheres do. Microparticles smaller than 10 μm administered intranasally are believed to be taken up by the M-cells overlaying the nasal-associated lymphoid tissue (NALT) and transported to sub-mucosal layers. However, in case of the nanoparticles, besides the M-cell-associated phagocytosis, the epithelial cells are also involved in the transport of nanoparticles by internalization. Recently, many studies confirmed that association of vaccines to the nanoparticulate systems has shown to enhance the antigen uptake by nasal lymphoid tissues.

Films

Mucoadhesive films may be preferred over adhesive tablets in terms of flexibility and comfort. In addition, they can circumvent the relatively short residence time of gels on the mucosa, which are easily washed away and removed by saliva. An ideal film should be flexible, elastic, and soft, yet adequately strong. It must also possess good mucoadhesive strength in order to be retained for the desired duration of action. Swelling of film, if it occurs, should not be too extensive in order to prevent discomfort. e.g. Chlorpromazine, chitosan/ pectin (Boddupalli B.M. 2010)

Patches

Patches are laminates consisting of an impermeable backing layer, a drug-containing reservoir layer from which the drug is released in a controlled manner, and a mucoadhesive surface for mucosal attachment. Patch systems are similar to those used in transdermal drug delivery. Two methods used to prepare adhesive patches include solvent casting and direct milling. In the solvent casting method, the intermediate sheet from which patches are punched is prepared by casting the solution of the drug and polymer(s) onto a backing layer sheet, and subsequently allowing the solvent(s) to evaporate. In the direct milling method, formulation constituents are homogeneously mixed and compressed to the desired thickness, and patches of predetermined size and shape are then cut or punched out. An impermeable backing layer may also be applied to control the direction of drug release, prevent drug loss, and minimize deformation and disintegration of the device during the application period. e.g. Insulin, chitosan/ PEG (Boddupalli B.M. 2010)

Gels and ointments

Semisolid dosage forms, such as gels and ointments, have the advantage of easy dispersion throughout the nasal mucosa. However, drug dosing from semisolid dosage forms may not be as accurate as from tablets, patches, or films. Poor retention of the gels at the site of application has been overcome by using mucoadhesive formulations. Certain mucoadhesive polymers, for example, sodium carboxymethylcellulose, carbopol, hyaluronic acid, and xanthan gum, undergo a phase change from liquid to semisolid. This change enhances the viscosity, which results in sustained and controlled release of drugs. Hydrogels are also a promising dosage form for nasal drug delivery. They are formed from polymers that are hydrated in an aqueous environment and physically entrap drug molecules for subsequent slow release by diffusion or erosion. The application of mucoadhesive gels provides an extended retention time in the nasal cavity, adequate drug penetration, as well as high efficacy and patient acceptability. Eg. Sulphadicramide, multiple polymers and Puerarin, poloxamer/ carbopol (Shaikh R. 2011)

7. Methods of production of nasal mucoadhesive formulations

Most nasal formulations on the pharmaceutical market today are in the form of liquid sprays, although dry powder formulations resulted in a higher drug bioavailability in several studies e.g. possibly as a result of increased residence time and improved deposition. The deposition of a dry powder formulation is determined by the particle size: particles smaller than 10 μm in diameter risk pulmonary deposition while those exceeding 50 μm might be deposited in the anterior part of the nose, which is covered by non-ciliated, stratified squamous epithelium and is unfavourable for absorption.

The usual method of production of mocoadhesive microspheres involves dissolution of the drug to incorporate it into the carrier, followed by lyophilisation or spray-drying. Although spray drying is less expensive than lyophilisation, both have some disadvantages with respect to drug stability, solvent residues and the size range of the resulting particles. An alternative could be to create interactive mixtures with a mucoadhesive carrier, if such mixtures can be obtained in the appropriate size range. Interactive, or ordered, mixtures are binary mixtures in which fine drug particles are adhered to coarse carrier particles to form interactive units, resulting in a highly homogeneous mixture. The fine particles are attached to the surface of the carrier particles mainly by van der Waals and electrostatic forces. For successful attachment, the carrier particles must be able to exert enough force to break up agglomerates of the small particles during the dry mixing process. This ability will primarily be determined by the density, surface roughness, shape, flowability and size of the carrier particles.

The concept of interactive mixing has been applied to formulations administered by inhalation, as an alternative to metered-dose inhalers. Because of the strong adhesive forces between the fine drug particles and the carrier particles, mixing times have been limited to prevent the strong attachment to the carrier particles resulting from adhesion to ‘‘active sites”.

8. Methods of evaluation (Tangri P. 2011)

Despite the intense focus surrounding mucoadhesive systems over the past few decades, no standard test methods have been specifically designed for mucoadhesion analysis. The majority of such testing techniques throughout the literature are found to be in vitro in nature. Despite this, there are a few in vivo experimental setups that have also been successfully implemented.

In vitro tests / ex vivo
- Methods determining tensile strength
- Methods determining shear stress
- Adhesion weight method
- Fluorescent probe method
- flow channel method
- Mechanical spectroscopic method
- falling liquid film method
- Colloidal gold staining method
- Viscometer method
- thumb method
- Adhesion number
- Electrical conductance
- swelling properties
- In vitro drug release studies
- mucoretentability studies

In vivo methods
- use of radioisotopes
- use of gamma scintigraphy
- use of pharmacoscintigraphy
- use of electron paramagnetic resonance(EPR)
- X ray studies
- Isolated loop technique

8.1 In vitro studies of mucoadhesion

In vitro tests are by far the most common techniques for the determination of polymers mucoadhesive properties.

8.1.1 Measuring the force of attachment

The most obvious method in which to assess a systems mucoadhesiveness is through the determination of the adhesive strength between polymer and the attached substrate. The adhesive strength at such a bonding interface can be measured by measuring the force required to detach one entity from the other through the application of an external force. As such the destruction of the adhesive bond is usually under the application of either a shearing, tensile or peeling force.

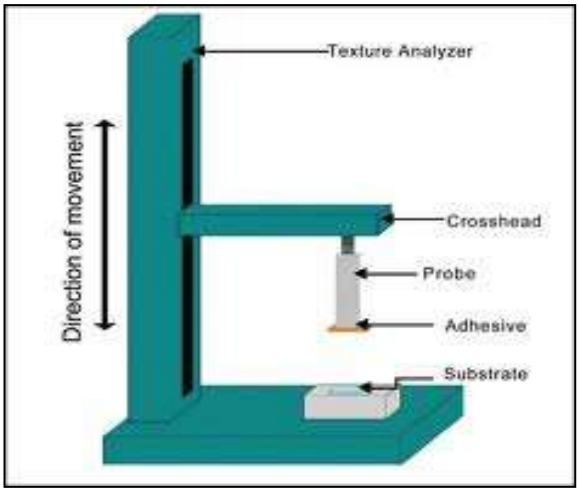

Fig.8 Texture profile analyzer in bioadhesion test mode (Shaikh R. 2011)

The determination of such a force of attachment was investigated by Smart et al. Here the authors used a modified version of the Wilhelmy plate surface technique in order to determine mucoadhesion of a range of candidate polymers. The device itself basically consists of a glass plate (which is laden with the polymer to be studied) suspended from a microbalance. The polymer-coated plate was then slowly dipped into a beaker of mucus. The work required removing the various polymer-coated glass slides could then be related to one another and their adhesiveness could be ranked. This technique had the advantage of allowing the analysis of mucoadhesion under different environmental conditions via simple modification of instrumental setup.

Subsequently though Mikkos and Peppas pointed out the short comings of such a technique due to the possible dissolution of the polymer upon testing. They suggested that this effect may be limited if polymer plates of the candidate material were used instead of polymer-coated glass plates. Further shortcomings were the lack of biological tissue in such a setup may not represent true mucoadhesion.

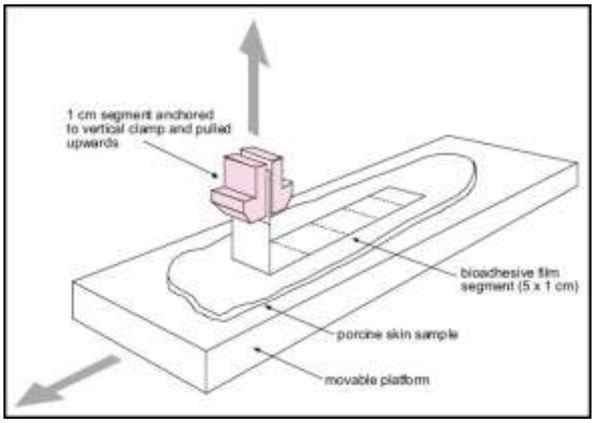
1 cm segment anchored to vertical clamp and pulled upwards
bioadhesive film segment (5 x 1 cm)
porcine skin sample
movable platform

Fig.9 Simplified representation of a typical test setup to determine peel strength of bioadhesive films (Shaikh R. 2011)

Despite the simplicity and efficiency of such a technique, tensile tests provide an incomplete picture of the process of mucoadhesion. It must be borne in mind thatmost mucoadhesive delivery systems will tend to exhibit other mechanical forces, such as the shear stresses exhibited within the nasal cavity or GI tract. The effect of both these important forces on measuring the adhesive bond was made possible via the use of a dual tensiometer.

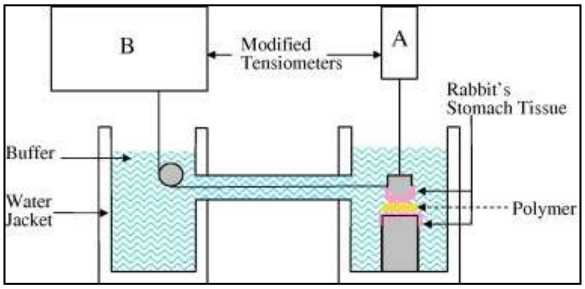

Fig.10 Modified tensiometers to test mucoadhesion

A method more recently employed by Grabovac et al. does in fact allow for the measurement of mucoadhesion under the application of a shearing stress. The test is centred on the use of a rotating cylinder, wherein the adhesion time of various compressed polymer discs to porcine small intestinal mucosa was established. The procedure itself involved attaching polymer discs to freshly excised porcine intestinal mucosa which had prior been spanned onto a stainless steel cylinder. The cylinder was then placed within a dissolution apparatus and rotated at a speed of 125 rpm. Changes in the test system were visually determined every 30 min and measured until all the discs were either disintegrated or detached from the mucosal surface (Andrews G.P. 2009).

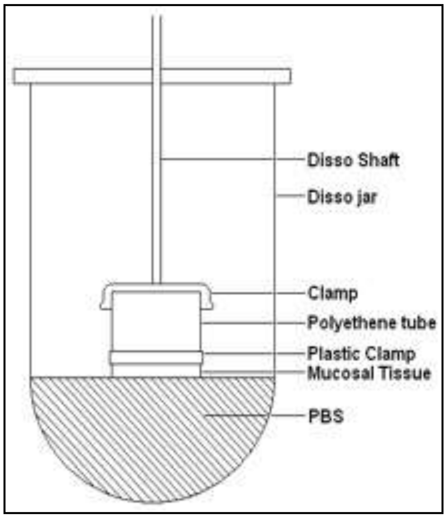

Fig.11 Rotating cylinder apparatus

8.1.2. Rheological measurement of mucoadhesion

Numerous authors have suggested that the rheological profiling of polymer–mucus mixtures can provide an acceptable in vitro model representative of the true in vivo behaviour of a mucoadhesive polymer. The mucoadhesive potential of polymer candidates was initially determined by rheologically comparing binary polymer/mucus blends to the rheological sum of similarly concentrated mono component mucus and polymer systems. Findings showed that the mucoadhesive polymer/mucus mixtures exhibited synergistic rheological profiles, the causes of which were attributed to bond formation between polymer and mucus culminating in an increase in total system structure.

The validity of such rheological measurements as a method of ranking mucoadhesive order was reinforced by the work undertaken by Tamburic and Craig. Within this study, the authors compared tensile mucoadhesive testing with shear rheological measurements using three polyacrylic acid-based polymers. They found that both measuring techniques gave the same rank order over adhesiveness for the polymers studied.

The isolation of fresh mucus for mucoadhesion analysis can be a laborious task. This has led many authors to turn their attention to the use of commercially dried mucus which is then re-hydrated before testing. Validation of the use of such mucus was carried out by Rossi et al. Within this study, investigators determined if there was in fact any variation in mucoadhesion testing through the use of different mucus-derived substrates. The authors rheologically studied the effect of two different freeze-dried porcine mucins and one type of freeze-dried bovine mucin on rheological parameters. Through their investigations, they discovered that the choice of mucus type was important in particular for the ionsensitive polyacrylic acids. In conclusion, authors stated that mucoadhesive polymers can be affected not only by the biochemical structure of glycoproteins but also by the way the mucin sample is prepared (Andrews G.P. 2009).

8.1.3 Shear stress method

The measurement of the shear stress gives a direct correlation to the adhesion strength. In a simple shear stress measurement based method two smooth, polished glass boxes are selected; one block is fixed with adhesive on a glass plate, which is fixed on leveled table. The level is adjusted with the spirit level. To the upper block, a thread is tied and the thread is passed down through a pulley, the length of the thread from the pulley to the pan is 12cms. At the end of the thread a pan of weight 17gms is attached into which the weights can be added.

A recent method involves the measurement of mucoadhesion by use of a stainless steel rotating cylinder which is coated with freshly excised porcine intestinal mucosa to which polymer discs are attached. The cylinder is placed in a dissolution apparatus and rotated at 125rpm. It is analyzed every 30mins for the attachment of the polymers discs (Tangri P. 2011).

8.1.4 Work of adhesion (Tangri P. 2011)

Another method used to measure the in vitro mucoadhesive capacity of different polymers is a modified method developed by Martti to assess the tendency of mucoadhesive materials to adhere to the mucosa.

Sheep intestine is removed and kept in tyrode solution at 4˚C; segments of 6-7cm long are cut from the intestine, the lower end tied to the glass tube of diameter 15mm. 6mm paracetamol plane tablets, paracetamol tablets layered on one side with mucoadhesive polymer, and the paracetamol in matrix tablets (2:1) ratio are prepared. A thread was passed through a fine hole drilled in the tablets to be tested and tied around the tablet. The other end of the thread is tied to the glass rod suspended from the stand. To the other end of the glass rod, a pan is tied in which a beaker was placed. After inserting the tablet into GI segment and lightly pressing the GI segment with tablet by a forceps, the assembly is kept undisturbed for 30mins to 1 hour. Then water is added to burette slowly drop by drop into the beaker. The amount of water required to pull out the tablet from the intestinal segment represents the force required to pull the tablet against adhesion.
$F=0.00981\ W/2$
W= amount of water.

8.1.5 Swelling studies (Tangri P. 2011)

Mucoadhesive dosage forms are weighed individually (W_1) and placed separately in Petri dishes containing 4ml of phosphate buffer pH 6.6 at regular intervals(0.5,1,2,3,4,5,6 hours). The dosage forms are removed from the Petri dishes and excess surface water is removed using filter paper. The dosage form are reweighed and swelling index (SI) is calculated as follows,

$SI= (W_2-W_1)/W_1$

8.1.6 In vitro drug release

These are performed in phosphate buffer pH 6.6, 150ml at 37˚C in a modified dissolution apparatus which consists of a 250ml beaker and a glass rod attached with a grounded glass disk (2cm dia) as a donor tube. The back surface is attached to the glass disk with an adhesive cynoacrylate adhesive. The donor tube is dipped into the medium and stirred at constant rpm. 5ml aliquots are withdrawn at preset times (0.08, 0.16, 1, 2, 3,4,5,6 hours), filtered through a 0.2 micron filter and absorbance measured at 290nm (Tangri P. 2011).

8.1.7 Novel electromagnetic force transducer technique

It is remote sensing instrument that utilizes a calibrated electromagnet to detach a magnetic loaded polymer from a tissue. It measures the adhesive force by monitoring the magnetic force required to exactly oppose bioadhesive force (Tangri P. 2011).

8.1.8 Tests for mucoadhesive microspheres (Tangri P. 2011)

Adhesion number- It is the ratio of the number of particles attached to the substrate to the total number of applied particles. It is expressed as a percentage.

Falling liquid film method- It as a quantitative, in-situ technique. In this method the percentage of particles which get retained on a mucosal tissue, spread on a plastic slide in an inclined position, when a suspension of the microspheres is allowed to flow down the tissue. The quantification can be done by the aid of coulter current method.

8.2 Other in vitro tests

Park and Robinson determined the effect of various polymer and mucin interactions via the use of fluorescent probes. The adhesion caused a change in the degree of fluorescent which was proportional to the polymer binding. Batchelor and co workers designed a technique in which fluorescently labeled alginate solutions of known rheological profile were delivered onto porcine oesophageal tissue. A washing solution to mimic flow was selected and it was seen that the 20% of dose remained in contact with the tissue for upto 30 mins (Tangri P. 2011).
Another imaging technique that did not involve fluorocence was developed by Kockisch and colleagues. Here investigators developed a semi quantitative image analysis based on the technque for the in vitro and in vivio detections of polymers with an affinity for the mucosal surfaces of the oral cavity.
Takaeuchi and co workers measured the mucoadhesion of different polymers via the BIACORE instrumentation. This system was based on the optical phenomenon of surface plasmon resonance (SPR) (Andrews G.P. 2009).

In Vitro drug release

To carry out *In Vitro* drug release, accurately weighed 50 mg of loaded microspheres are dispersed in dissolution fluid in a beaker and maintained at 37±2° C under continuous stirring at 100 rpm. At selected time intervals 5 ml samples are withdrawn through a hypodermic syringe fitted with a 0.4 µm Millipore filter and replaced with the same volume of pre-warmed fresh buffer solution to maintain a constant volume of the receptor compartment. The samples are analyzed spectrophotometrically. The released drug content is determined from the standard calibration curve of given drug (Parmar H. 2010).

In Vitro diffusion studies

In Vitro diffusion studies are performed using in vitro nasal diffusion cell. The receptor chamber is filled with buffer maintained at 37±2°C. Accurately weighed microspheres equivalent to 10 mg were spread on sheep nasal mucosa. At selected time intervals 0.5 ml of diffusion samples were withdrawn through a hypodermic syringe and replaced with the same volume of prewarmed fresh buffer solution to maintain a constant volume of the receptor compartment. The samples were analyzed spectrophotometrically (Parmar H. 2010).

Stability studies

The preparation is divided into 3 sets and was stored at 4°C (refrigerator), room temperature and 40°C (thermostatic oven). After 15, 30 and 60 days drug content of all the formulation was determined spectrophotometrically (Parmar H. 2010).

Drug polymer interaction (FTIR) study

IR spectroscopy can be performed by Fourier transformed infrared spectrophotometer. The pellets of drug and potassium bromide are prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra are scanned in the wave number range of 4000- 600 cm-1. FTIR study was carried on pure drug, physical mixture, formulations and empty microspheres (Parmar H. 2010).

8.3 In vivo studies

Due to cost, time constraints and ethical considerations, in vivo mucoadhesive studies are less commonly seen in the literature than in vitro testing. Despite these concerns in vivo testing is still the important if the true mucoadhesive potential of a system is to be determined. As such in vivo techniques have found there most extensive use in the analysis of potential oral mucoadhesive dosage forms (Andrews G.P. 2009).

The development of a non-invasive technique to determine the transit of mucoadhesive polymers was undertaken in the work by Davis. Here, the transit of polymers could be imaged via labeling of the mucoadhesive polymer system with a c-emitting nucleotide. Detection of the polymer systems was then determined via c-scintigraphy.

Finally a more recently employed non-invasive imaging technique was presented by Albrecht et al. Investigators here used magnetic resonance imaging to localise the point of release of thiolated polymers from dosage forms via the use of gadolinium. In vivo mucoadhesion was determined by ascertaining the residence time of the fluorescently-tagged thiomer on intestinal mucosa of rats after 3 hours. This technique allowed the comparison of mucoadhesive properties of candidate polymer systems in vivo.

9. Nasal drug delivery opportunities

Exploitation advantages of administering drugs nasally compared to oral or parenteral route could lead to a fast track product development. This is proven by the increasing number of nasally administered drugs as well as companies either entirely specialized in NDD or have strong presence in NDD research.

The possibility of increased drug absorption will allow product development of nasal peptides and small proteins, while this would not be possible with the oral route and eliminates the use of injections, with its widely published limitations. The increased absorption (due to high epithelial permeability/porosity) together with low enzyme activity will act synergistically towards increasing drug absorption. Controlled release can be achieved also after nasal administration. A lot of the polymers used in nasal mucoadhesion have been thoroughly tested in humans for other application and most have GRAS (Generally Regarded As Safe) status already. While this does not rule out the need to perform local nasal toxicological studies, it reduces significantly the cost of product development thereby making this route of administration more appealing to industrial executives.

Apart from expanding product portfolio, it is an economical way of life cycle management. Converting a boldQ drug previously administered parenterally to a nasal product will not only expand/extend patent position but also decrease cost of goods and increase patient compliance. The short product development trajectory and multiplicity of disease states where NDD can be applied are the other advantages that need to be mentioned. The dual strategy of reducing product development time by applying existing excipients for NDD has been successfully applied in the development of products such as Rhinocort (Ugwoke M.I. 2005).

10. Safety considerations

Absorption enhancement and toxicity are the key issues in the search and design of effective and safe drug formulations for the nasal route. From pharmacokinetic point of view, achieving sustained release for several hours is desirable. However, toxicity of the excipients could be aggravated because of extended contact with the nasal mucosa. Ideally, mucoadhesive polymers or other excipients should be cleared from the nasal mucosa within a few hours in order not to impair the mucociliary system. Most mucoadhesives act to oppose the mucociliary transport system via prolongation of intranasal polymer– drug residence time. Depending on the intrinsic properties of a particular polymer and the drug loaded in it, this could result in higher toxicity compared to simple nasal solutions or aerosols.

The major toxicological targets for nasally administered drugs include local irritation/tissue damage, epithelial/subepithelial toxicity and ciliotoxicity. Furthermore, the relatively low mucus turnover of the nasal mucosa compared to the gastrointestinal mucosa, extended mucoadhesion could result in a fertile environment for establishment of opportunistic respiratory tract infections. When developing mucoadhesive drug products for the nasal route, it is pertinent to assess the effect of both the drug and excipients on these targets.

Among all the mucoadhesives used for nasal drug delivery, none has undergone extensive mucosal and mucociliary toxicity studies like the chitosans. For the ex vivo studies, a range of chitosans with different molecular weights were applied to freshly amputated human nasal turbinates, and their effect on mucus transport rate was recorded. The larger the molecular weight of the polymer, and the more volume applied led to longer depression of the mucus transport rate. The transient inhibitory effect on turbinate mucus transport rate correlated with the volume of chitosan solution applied and the molecular weight of the chitosan tested.

Considering the nasal toxicity data available in literature and efficacy as nasal mucoadhesives, chitosans and some cellulose derivatives appear more promising for developing nasal drug products. Other mucoadhesives could also be very useful; however more studies are necessary, especially investigations on the effects of the excipients on ciliary beat frequency and/or mucociliary transport system. This is very important because the concept of nasal mucoadhesion opposes the basic physiological system (mucociliary clearance) that protects the respiratory system from bacteria, viruses and noxious substances (Ugwoke M.I. 2005).

There are a lot of exciting developments in the field of NDD including mucoadhesion. Newly marketed products based on existing polymers are on the increase, while new polymers and administration devices are still being developed. There is a lot of ground for optimism with respect to benefits derivable from more fundamental research and application leading to better understanding of the subject and eventually more marketed products. A point of caution will always be the safety aspects of nasal products, although the recent developments of both in vitro and in vivo models is a big boost to speeding up clinical developments and eventually time-to-market of new products (Ugwoke M.I. 2005). In the near future we should expect to see a range of novel nasal products reaching the market. It is envisaged that these products will in the first instance primarily comprise products for crisis treatment such as treatment of acute pain, panic attacks, sleep from induction, erectile dysfunction, nausea, heart attacks, Parkinson’s disease, etc.

Further in the future we should also see improved nasal products for treatment of long term illnesses such as diabetes, growth deficiency, osteoporosis, endometriosis, fertility treatment. At present the required drugs are usually either given by injection, have a not totally appropriate plasma profile or display unwanted pharmacological effects due to inappropriate dosing regiments. In the area of vaccines the future should also see a range of novel nasal vaccines introduced to include DNA based vaccines especially for vaccination against respiratory diseases. These vaccines will provide better protection against infection and the possibility of a wider and safer use in third world countries.

Finally, it will be interesting to follow the developments in the area of nose to brain delivery and to ascertain whether it will prove possible to control and enhance the use of the route of delivery for treatment of diseases where rapidly achieved and high concentrations of drug in the brain are essential for an efficient therapeutic response (Illum L., 2003).

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MUCOADHESIVE DRUG DELIVERY SYSTEM – RECTAL

Kulkarani Akshada, Pawar Yogesh

Need of rectal drug delivery system:

Administration via the per oral route is the most commonly targeted goal of new drug and dosage form research and development, oral administration is not always feasible or desirable. The potential for oral dosage form development is severely limited for active agents that are poorly absorbed in the upper gastrointestinal (GI) tract and unstable to proteolytic enzymes. Some agents cause local stomach or upper GI irritation or require doses in excess of 500 mg. Certain patient populations, notably children, the elderly, and those with swallowing problems, are often difficult to treat with oral tablets and capsules. Additionally, treatment of some diseases is best achieved by direct administration near the affected area, particularly with diseases involving ophthalmic, otic, dermal, oral cavity, and anorectal tissues. Although oral administration can be used for drugs targeted for some of these diseased tissues, exposure of the entire body compartment to the administered drug is inefficient and can lead to undesired adverse effects. (Singh K.K. 2005) Rectal drug administration is used in situations when patients are vomiting or suffering from nausea. The first-pass elimination of drugs is also partially avoided by rectal administration and furthermore the rectum environment is quite constant with respect to pH; composition, volume and viscosity of fluid; and less influenced by food. This offers the opportunity for rate controlled rectal drug delivery. (Sunena 2011) Rectal drug administration is amenable, however, to both local and systemic drug delivery. It has been effectively utilized to treat local diseases of the anorectal area as well as to deliver drugs systemically as an alternative to oral administration. (Singh K.K. 2005)

Physiology of human rectum:

Human rectum is the terminal 10-15 cm slightly dilated part of large intestine. In the resting state, the rectum does not have any active motility. Normally the rectum is empty and contains 2-3 mL of inert mucus fluid. This mucus has no enzymatic activity or buffering capacity. There are no villi or microvilli on rectal mucosa and thus a very limited surface area is available for absorption.

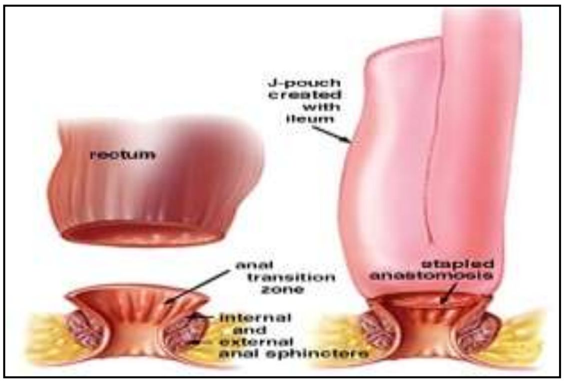
J-pouch created with ileum
rectum
anal transition zone
stapled anastomosis
internal and external anal sphincters

Fig1: Parts of rectum

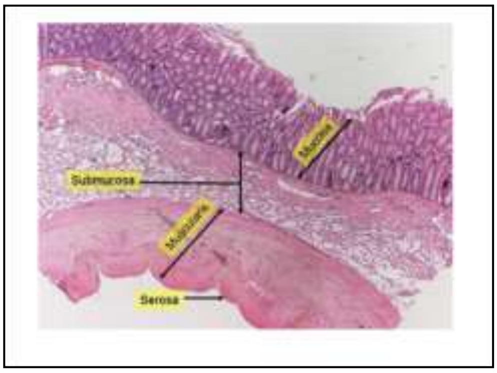

Fig2: T.S. of rectum

Fate of drug in rectum:

Fate of drug absorbed from the rectum depends upon the position of it in the rectum. Both blood and lymphatic vessels are abundant in sub mucosal region of rectal wall. Upper haemorrhoidal vein drains in to portal circulation in the upper region will pass through the liver before entering the systemic circulation, while the middle and lower haemorrhoidal veins drain directly in to the inferior venacava. So the drugs absorbed in the lower region of the rectum will directly enter in to the systemic circulation. (C. A. N n.d.)

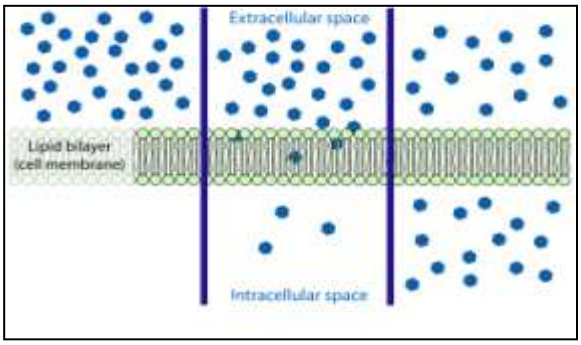

Fig3: Mechanism of simple diffusion

Rectal absorption is a simple diffusion process through the lipoidal membrane in which carrier-mediated mechanisms play no role. The rates of absorption increase with increasing solubility and release of drugs by vehicles or excipients, although drug-excipient interaction is related to the decreased absorption rate; membrane-active excipients promoting absorption are more effective in the colorectal area. (Baria A H 2009), (Patil B 2011) Bioavailability of this route depends upon pH of environment, lipid solubility of drug. (Patil B 2011)

Advantages and disadvantages of rectal drug delivery system: (Rytting J. H. n.d.)

Advantages-

- Improved enzymatic drug stability-

Many proteolytic and other enzymes in the stomach and small intestine result in drug degradation which prevents effective absorption following oral administration. Therefore many drugs that cannot be administered effectively orally can be administered rectally without as much enzymatically catalyzed degradation.

- Partial avoidance of hepatic first pass-

It is usually reported that the inferior and middle rectal veins drain into the inferior vena cava. This allows drugs absorbed by this route to partially bypass the portal system and the associated first-pass metabolism in the liver, suggested as an advantage of rectal administration of insulin over parenteral administration.. Studies in rats suggest that first pass metabolism is almost totally avoided by rectal administration.

- Higher drug load-

Oral administration in a single tablet is limited to about 1 g. Typically, suppositories intended for adults can be as large as 2.5 g. This should allow for two to three times higher drug loads.

- Lymphatic delivery-

Some studies have indicated that significant amounts of some drugs are transported through the lymphatic systems following rectal administration. Lymphatic transport can further be enhanced by using different absorption enhancers in the formulation.

- Constant and static environment-

Orally administered drug must usually pass through a series of diverse environments prior to absorption (transit through the stomach and the small intestine and perhaps to the large intestine and colon) depending on the drug and the absorption siteleading to complicated set of condition. Compared to the oral route of administration, the rectal route provides a much more constant environment for the drug as it is absorbed.

- Patients with swallowing difficulty-

For a number of patients, especially children and elderly, problems associated with swallowing and nausea when taking oral medication can be largely obviated by rectal administration.

- Avoidance of overdosing-

Certain drugs, such as sedatives, on oral administration may raise a concern with respect to the possibility of severe accidental or intentional overdosing. This danger is practically eliminated by rectal administration.

Disadvantages-

- Patient acceptance and compliance-

In some cultures there is reluctance by many to consider rectal administration. This has resulted in a tendency by pharmaceutical market to avoid rectal dosage forms, except for the most obvious indications and situations where other dosage forms have substantial disadvantages.

- Potential for non-specific drug loss-

There are at least two common problems that can lead to drug loss following rectal administration. First, for effective absorption, the dosage form must be retained in the rectum. Thus if the dosage form or parts thereof are lost prematurely from the rectum, drug absorption will be substantially reduced. Second, there is the possibility that the drug or some important excipients may interact with constituents of fecal matter or material fluid present in the rectum. This may reduce the drug absorption and diminish effectiveness.

- Limited fluid in the rectum-

The amount of liquid in the rectum (2-3mL) is small compared to the volume of fluid available throughout the GI tract when a drug is administered orally. Hence drug dissolution might be the rate determining step.

- Formulation-

There are a number of formulation variables such as melting and liquefaction can lead to difficulties in rectal drug absorption. The pK_a of the drug, the pH of the rectal fluids, the presence of buffers, and the buffer capacity of the rectal fluid as well as the partition coefficient of the drug influence drug absorption and must be considered in the formulation of a suppository or other rectal dosage form. Storage temperature, time, and conditions can have a profound effect on both the stability and release characteristics of a drug from a rectal dosage form. Each of these considerations lead to potential difficulties in the formulation, manufacture and distribution of rectal dosage forms.

- Expense-

Suppositories and other rectal dosage forms are more expensive to prepare and dispense than simple tablets. Therefore, unless there is a significant need, suppositories are not likely to be used.

Factors affecting drug absorption:

The dose of a drug administered rectally may be greater than or less than the dose of the same drug given orally, depending on such factors as the constitution of patient , the physicochemical nature of drug and its ability to travel through the physiological barriers to absorption, the nature of vehicle and its capacity to release drug and make it available for absorption.

Factors that affect rectal absorption of the drug may be divided in to two main groups ; physiological factors and physicochemical factors of the drug and the vehicle. (Allen L V 2005)

Physiological factors:

Among the physiologic factors that affect the drug absorption from the rectum are the colonic contents circulation route and the pH and lack of buffering capacity of the rectal fluids. (Allen L V 2005)

- Colonic content-

For systemic effect greater absorption may be expected from the rectum that is void. A drug will obviously have greater opportunity to make contact with the absorbing surface of the rectum and colonin an empty rectum. (Allen L V 2005)

- Circulation route-

Drugs absorbed rectally bypass the portal circulation during their first pass into the general circulation thereby enabling drugs to exert systemic effects. The lower haemorrhoidal veins surrounding the colon receive the absorbed drug and initiate its circulation throughout the body, bypassing the liver. Lymphatic circulation also assists in the absorption of rectally administered drugs. (Allen L V 2005)

- pH partition-

Rectal fluids are essentially neutral in pH 7-8 and have no effective buffer capacity , the form in which the drug is administered will not generally be chemically changed by the environment. (Allen L V 2005) Drugs are best absorbed through the rectal mucosa in their un-ionized or neutral form. Drugs with high partition coefficients (more lipophilic) tend to be better absorbed.[3]

Physicochemical factors:

Physicochemical factors include such properties as relative solubility, particle size, charge, spreading of the administered formulation. (Allen L V 2005)

- Solubility-

Lipid water partition coefficient of a drug is an important consideration in the selection of a vehicle and in anticipating drug release from that vehicle. Drug solubility also affects the choice of suppository base or other vehicle. Generally, the drug should have little tendency to remain in the vehicle upon melting or dissolution. Therefore, it is usually suggested that water soluble drugs are best delivered from fatty vehicles and that more lipophilic compounds from water-soluble vehicles. (Rytting J. H. n.d.)

- Particle size-

The smaller the drug molecule, the more readily it can be absorbed. For larger molecules, some type of facilitated transport or the use of penetration enhancers have been found to increase drug absorption from the rectum as well as from other delivery routes. (Rytting J. H. n.d.)

- Charge-

Charged molecules have been found to pass through the rectal mucosa less effectively than neutral molecules in most cases. This can sometimes be overcome by modifying the pH or allowing the charged species to interact with another molecule or ion that helps neutralize the charge. (Rytting J. H. n.d.)

- Spreading of the formulation-

For optimal drug absorption, it is important that the suppository or vehicle melts or dissolves rapidly and spreads over the rectum walls. Thus the rheological behaviour of the vehicle can have a significant effect on the release of the drug and the ability of the drug to come into contact with the rectal mucosa. (Rytting J. H. n.d.)

Types of dosage forms:

Solid:

Solid suppositories are the most common dosage form used for rectal drug administration and represent greater than 98% of all rectal dosage forms. Suppositories are the solid dosage forms intended for insertion in to body orifices where they melt, soften, or dissolve and exert local or systemic effects. Suppositories are commonly used rectally and vaginally and occasionally urethrally. (Rytting J. H. n.d.)

E. Bergogne-Bérézin*a et. al.* Studied the influence of the pharmaceutical formulation of suppositories on the rectal absorption and systemic distribution of ß lactams and aminoglycosides. (E. Bergogne-Bérézina 1999)

The shape and size of the suppository must be such that it can be easily inserted in to the intended orifice. Suppositories composed of fatty bases (low-melting) or water soluble bases (dissolving) which vary in weight from 1 g (children) to 2.5 g (adult). Lipophillic drugs are usually incorporated into water-soluble bases while hydrophilic drugs are formulated into the fatty base suppositories, this maximizes removal of the drug from the suppository base to the environment of the rectal cavity. For suppositories made from fatty bases, melting should occur rapidly near body temperature (37°C). Water-solublesuppositories should likewise readily dissolve at 37_C to facilitate drug release and subsequent absorption. With both fatty-base and water-soluble suppositories, the potential effects of incorporated drug on melting or dissolution properties need to be evaluated. (Rytting J. H. n.d.)

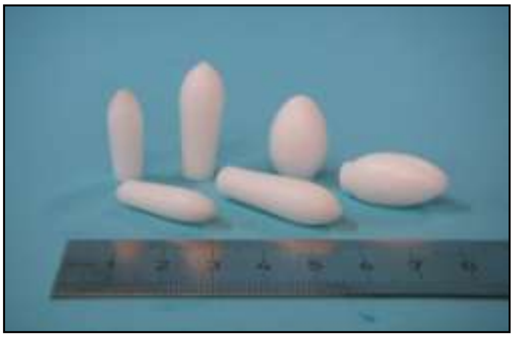

Fig4: Shapes of suppositories

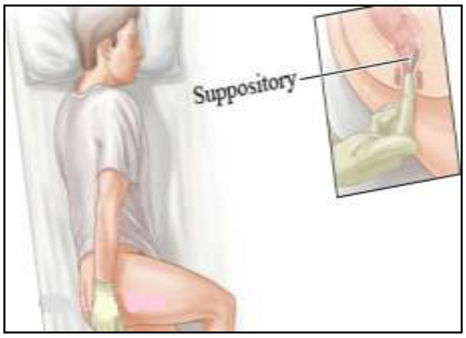

Fig5: Insertion of suppository

Semi solid:

Rectal ointments-

It is used for the symptomatic relief against anal and peri-anal pruritus, pain and inflammation associated with hemorrhoids, anal fissure, fistulas and proctitis. Rectal ointment should be applied several times in a day according to the severity of the condition. For intrarectal, use, apply the ointment with the help of special applicator. (Patil B 2011)

Liquid:

Solutions, suspensions, or retention enemas represent rectal dosage forms with very limited application, largely due to inconvenience of use and poor patient compliance. In many cases, these formulations are utilized to administer contrast media and imaging agents for lower GI roentgenography. (Rytting J. H. n.d.)

Retention enemas are used for local or systemic effects. The drugs like hydrocortisone and aminophylline are used in this dosage form. (C. A. N n.d.)

Evacuation enemas are used for cleansing of bowel. (C. A. N n.d.)

Fig6: Enema applicator

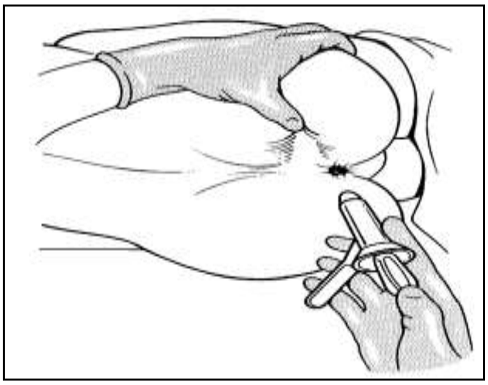

Fig7:Insertion of enema

Controlled release dosage forms: (Singh K.K. 2005)

Controlled release suppositories-

1. Suppositories containing fatty base-

Fatty bases have been popular because of their non toxicity and low irritation profile. Cocoa butter or theobroma oil was the first modern base used. Semi synthetic fatty bases are composed of triglycerides derived from palm. They are stable with low irritation potential.

- Combination of mixture of hard fats-

Drug release from suppositories can be controlled by mixing various types of hard fats having higher melting points. Hard fat (witepsol) used as a base for suppository consist of mixture of triglyceride, diglyceride and monoglyceride.

- Suppositories containing sugar esters-

Sucrose fatty acid ester has been used as an additive in the suppositories to improve the disintegration of suppositories and to reduce the irritation of rectal mucosa. Incorporation of sugar esters in suppositories not only sustains the release of drug but also reduces the Crystallinity of drug, making it more stable.

Toshihito Takatori *et. al.* designed controlled-release morphine suppositories containing polyglycerol ester of fatty acid. The addition of PGEF to fatty suppository base Witepsol H15 resulted in a decrease of morphine release rate from suppositories. (TAKATORI T 2005)

- Suppositories containing lecithin-

Soya lecithin is a phospholipid and with its emulsifying properties can act as a drug release modifier.

- Suppositories containing polymeric spheres-

These systems consist of drug embedded in polymeric spheres, which are incorporated in suitable base. Drug is released from the spheres in controlled fashion, thus providing a sustained release effect. The polymeric nanospheres are in corporate into conventional triglyceride base, which melts in the rectum on insertion to release the nanosphere. The nanospheres are transported across the capillary membranes into the systemic circulation whereby they release the drug in controlled fashion.

- Suppositories containing phospholipon-

Suppositories containing witepsol W35 and phospholipon 90Gafter meltingand on contact with water or physiological aqueous media exhibited transformation into semisolid system of liquid crystalline micro structure.

- Suppositories containing crudlan-

Crudlan, a natural ß-1, 3-glucan has potential to be used in preparation of sustained release suppositories.

2. Controlled release suppositories containing polyethylene glycol-

Polyethylene glycol bases are water soluble bases used widely in the formulation of conventional suppositories. These bases generally dissolve slowly in limited body fluids and the release of the active ingredients from these bases is a function of the dissolution of the suppository base rather than melting.

Sustained release rectal gels-

Rectal gels have been primarily used for local action either externally or for internal application.

Aqueous polyacrylic acid gels are useful as a base for rectal drug administration. These gels can be adjusted to a suitable pH and viscosity, which have a definite effect on the drug release.

Eudragits, block co-polymer of methacrylic acid and methyl methacrylates in different ratios. These have been used either as aqueous gels for rectal administration.

Hydrogel based rectal drug delivery system-

Hydrogels are defined as macro molecular networks that swells but do not dissolve in water. Aqueous insolubility of hydrogel results from the cross links between adjacent macromolecules. The water swollen hydrogels with high degrees of swelling can provide rate controlling barriers to the diffusion of water soluble drugs thus allowing the highest attainable fluxes from polymers.

Polyvinyl alcohol hydrogels containing phospholipids prepared by low temperature crystallization method have been designed as trans rectal delivery system. The drug release profile from such hydrogel preparation shows Fickian diffusion.

Mucoadhesive dosage forms: (Baria A H 2009)

Bioadhesion is the state in which two materials, at least one biological in nature, are held together for an extended period of time by interfacial forces. In case of mucoadhesion, the biological tissue is the mucous membrane.

Factors to be considered for mucoadhesion-

- Polymer related factors
    a. Molecular weight
    b. Concentration of active polymer
    c. Flexibility of polymer chain
    d. Spatial conformation
- Environment related factors
    a. Applied strength
    b. pH
    c. Initial contact time
    d. Swelling
- Physiological veriables
    a. Mucin turnover
    b. Disease state

Mucoadhesive suppositories-

Drugs causing local irritation are formulated in mucoadhesive liquid suppository since conventional suppositories may exhibit some disadvantages. Rectal administration of conventional suppositories may cause local irritation and mucosal damage due to the properties of that can cause rectal bleeding on administration. This may lead to the feeling of alien, discomfort and refusal thus, lowering patient compliance. To solve the problems of conventional suppository, an attempt was made to develop an intelligent formula, mucoadhesive poloxamer based liquid suppository. It exists as a liquid at room temperature and turns into a gel instantly at physiological temperature, also, mucoadhesive polymers adhere the suppository to the rectal tissues which prevents its migration to the end of the colon. (Ramadan S M 2009)

Polymer	Bioadhesive property
CMC Sodium	+++
Carbopol 934	+++
Polycarbophil	+++
Tragacanth	+++
Poly(acrylic acid/divinyl benzene)	+++
Sodium Alginate	+++
Hydroxy Ethyl Cellulose	+++
HPMC	+++
Gum Karaya	++
Gelatin	++
Guar Gum	++
Thermally Modified Starch	+
Pectin	+
PVP	+
Acacia	+
Psyllium	+
Amberlite-200 resin	+
Hydroxy Propyl Cellulose	+
Chitosan	+

+++-Excellent, ++-fair, +-poor

Table1: Mucoadhesive polymer and there bioadhesive property (Yadav V K 2010)

Insitu gelling and mucoadhesive solution and suppository-

From an industrial viewpoint, solid suppositories are inconvenient to manufacture and handle since a heating process is required for melting the suppositories and filling them in a vessel. The vessel needs to be packaged together to maintain the shape of suppositories until administration. To solve the problems of conventional solid suppositories, it would be desirable to develop a liquid suppository which: (1) forms a gel at body temperature; (2) has a suitable gel strength not to be leaked out from the anus after administration; and (3) has a suitable bioadhesive force so as not to reach the end of the colon. (Jadhav U G 2009)

Drugs having extensive first pass effect are formulated in mucoadhesive suppositories in an attempt to improve its bioavailability and possibility restrict its absorption to only the lower Rectum, rectal solution with gelation at temperature range of 30-37°C. (Abd ElHady S S 2003)

If the absorption of drug across the colon and rectum is similar to the oral route, then it is suggested that suppository formulations are feasible. (Jadhav U G 2009)

Increasing the concentration of mucoadhesive polymers retards the release of drug from the suppository. Hence concentration of polymer added in formulation is important. (Abd ElHady S S 2003)

Mucoadhesive hydrogel-

Hydrogels are composed of three-dimensional network of hydrophilic polymer chains that could be cross-linked by chemical or physical bonding. They physically entrap drug molecules for subsequent slow drug release by diffusion or erosion depending on their state of hydration. For highly hydrated hydrogels, the drug diffusion occurs through the pores of the gel network, whereas, lower hydration allows drug to dissolve in the polymer and transport between the chains. The polymers cross-linking was reported to increase the hydrophobicity of the gel and diminish diffusion rate of the drug, whereas its soft rubbery nature minimizes mechanical and frictional irritation to the surrounding tissues. These characteristics of hydrogels, as well as their biocompatibility and increased duration of action, could be considered a potentially beneficial approach in increasing therapeutical efficiency of rectal forms of drugs. Liquid suppositories (hydrogels) that exist as liquid *in vitro* but gel *in vivo* have been proposed as alternatives to conventional suppositories. Ibuprofen, quinine, diazepam, and propranolol are examples of drugs presented as rectal hydogels to improve drug bioavailability or patient compliance. (El-Leithy E S 2010)

Evaluation of dosage form:

1. Measurement of gel strength-

The gel strength was determined according to the method adopted. 50 g of liquid suppository was put in a 100 ml graduated cylinder and gelled in a thermostat at 37°C. The apparatus for measuring the gel strength (weight 35 g) was then placed into the liquid suppository. The gel strength was determined by the time in seconds the apparatus took to penetrate 5 cm down through the gel. (Ramadan S M 2009) (Koffi A A 2008)

2. Mucoadhesion studies-

A hydrogel layer (10 mm in height, contact surface: 3.8 cm2) was placed in contact with a fragment of freshly excised rabbit or rectal mucosa under thermostated conditions, thus forming an adhesive joint between the two surfaces. After a preset contact time (10 min) under an initial contact strength, the two surfaces were separated at a constant displacement rate of 1 mm/s using a texture analyser. The strength was recorded as a function of the displacement, which allowed determining the maximal detachment force (Fmax) and the work of adhesion (W) which was calculated from the area under the strength displacement curve. (Koffi A A 2008) (Koffia A A 2006)

3. Measurement of gelation temperature-

Gelation temperature was assessed using the tube tilting method23. 2 ml aliquot of gel was transferred to test tubes, immersed in a water bath at 4° and sealed with aluminum foil. The temperature of water bath was increased in increments of 1° and left to equilibrate for 5 min at each new setting. The samples were then examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting through 90°. (KENY R C 2010)

4. *Invitro* evaluation-

For assessing the absorption potential of specific drug candidates or conducting studies evaluating correlations between drug structure and transport, in vitro models may provide the best approach. Both the tissue-diffusion cell systems and cultured colon cell lines have proved to be particularly useful. Diffusion chambers of various designs (e.g., Ussing chambers, Sweetana–Grass diffusion cells) are used to evaluate the permeation properties of drug candidates. These systems offer a distinct advantage that drug solutions may be added to the donor compartment under a variety of conditions (e.g., varying concentrations, pH, excipients). Samples are withdrawn periodically from a receiver compartment and analyzed for drug and metabolite content to obtain a true measure of permeation across the cellular barrier. In establishing procedures for these diffusion cell studies, since underlying muscle layer is not a barrier, the underlying muscle layer can be teased away and only the intact mucosal cell layer mounted in the diffusion chamber. This model may overestimate drug permeation unless great care is taken to maintain the mucus layer. Conversely, the use of mucolytic agents can be employed to ensure a mucus-free cell layer if true cellular drug permeability is to be determined. Work in the author's laboratories has demonstrated that the Sweetana–Grass diffusion cell model with muscle-free mucosal layers is reasonably resistant to experimental conditions. Excipients such as DMSO or ethanol can be utilized up to 10% (v/v) in order to improve solubility of drugs whose absorption may be limited by poor aqueous solubility. These muscle-free mucosal strips can be maintained with good viability for up to 120 min at 37°C. It hs to be ensured that tissue damage is not affecting drug transport values. (Rytting J. H. n.d.) (Baria A H 2009)

Another in vitro model which may provide useful information relative to rectal or colonic drug absorption is cultured cell monolayers. HT-29 and Caco-2 cells are two cell lines commonly employed for such studies. Both are human colon-cancer cell lines which can be grown on membrane filters and readily form confluent monolayers with intact tight junctional complexes can be used in experimental designs similar to the diffusion cell models. These cells are non-mucus secreting cell populations which resemble fetal small intestine in many of their metabolic properties. These models are useful for quantifying drug transport across colon cell lines. Care must be taken in interpreting data from transport studies which show parent drug metabolism during the absorptive phase since this may or may not also occur in vivo. (Rytting J. H. n.d.)

5. *Invivo* evaluation-

Rat model-

Preliminary in vivo evaluation of the rectal absorption potential of drug candidates is most easily achieved in a rat model Animals can be maintained at surgical plane anaesthesia for 120–180 min, and drug solutions or suspensions can be directly instilled into the rectal cavity. Instillation of approximately 0.25 ml is readily achieved in rats without loss of fluid from the rectal compartment. Moderate pressure clamps can also be used to prevent leakage of administered solutions from the rectal cavity. It is advisable to fast animals for 12–16 h prior to study to void the colon and rectal cavity of fecal material which may interfere with administration of the solution or absorption of the drug itself. Micro suppositories can also be evaluated in the rat model by utilizing specially designed molds to prepare small dosage forms, but care must be taken to guarantee drug content uniformity in these small devices. (Rytting J. H. n.d.)

Rabbit model-

Rabbits have been another common animal model utilized for examining rectal drug absorption. A possible drawback to the rabbit model is evidence suggesting that the rabbit colon is somewhat more ‘‘leaky’’ than the colon in other species. In this regard, the rabbit model may overestimate the extent of drug absorption achievable for small, water-soluble compounds which utilize intercellular transport pathways via water flux. (Rytting J. H. n.d.)

Dog model-

For evaluation of dosage forms designed and formulated for human use, the Beagle dog presents an ideal animal model. Solid suppositories (up to 2.5 g total weight) or micro enemas (up to 5 ml volume) can be readily tested in Beagles without concern for loss of dosing vehicle from the rectal compartment. Since the physical dimensions of the dog rectum approximates those of the human rectum, variables such as spreading of suppository bases can be reasonably well evaluated. The dog is obviously a convenient model for blood sampling and pharmacokinetics and is, therefore, well suited for the evaluation of rectal dosage forms. Studies from the author's laboratories and others have shown that dosage-form performance criteria generated in Beagle dog models are accurate predictors of performance in human clinical trials. (Rytting J. H. n.d.)

Role of penetration enhancer and solubilising agent:

Penetration enhancer-

Penetration enhancers are the excipients that improve drug solubility and stability in the formulation and enhance the permeation of the drug across the mucous membrane. Strong chelating agents and phenothiazines appear to enhance the rectal absorption of both low and high molecular weight compounds with a constant ratio of absorption via a paracellular route. Diethylmaleate enhances rectal absorption of low molecular weight compounds via a transcellular route. Various salicylates, diethyl ethylene malonate, and various fatty acids have been found to enhance both the paracellular and transcellular routes of absorption. In some respects, rectal drug administration is optimally suited for coadministration of drug entities with absorption-enhancing agents. (Rytting J. H. n.d.)

Solubilising agent-

It would be expected that solubilising agents may increase the rate of drug release from a suppository base by increasing the dissolution rate and perhaps by modifying the viscosity and interfacial tension of the vehicle with the rectal fluid. In addition to the effect that a solubilising agent or a surfactant can have on the drug and the vehicle, the surfactant may also have an effect on the mucous coating of the rectal membrane. This may increase absorption by reducing the thickness of the layer through which the drug must traverse or it may act as a penetration enhancer by increasing the permeability of the membrane through damaging the rectal mucosa. (Rytting J. H. n.d.)

Marketed products: (C. A. N n.d.)

Suppositories-

DULCOLAX-CIBA (bisacodyl)
PANADOL

Fig8: DUCLOX suppositories

CANASA-AXAN SCANDIPHARM (mesalamine)
NUMORPHAN-ENDO (oxymorphane)
ANUSOL HC-WARNER_LAMBERT (htdrocortisone)

Fig9: PANADOL suppositories

Aerosols-

PROCTOFOM HC (Schwarz)
CORTIFOM (Alaven)

Rectal administration of therapeutic agents represents a narrow part of the total pharmaceutical approach to disease management but it holds promise for increasing applications. Even though few countries may find rectal dosage form more acceptable these still represent small area of the worldwide market share which can be assigned to the rectal drug therapy.

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MUCOADHESIVE DRUG DELIVERY SYSTEM-VAGINAL

Deshpande Tanvee, Shinde Rohit, Pawar Yogesh

INTRODUCTION:

The vagina provides a promising site for local effect as well as systemic drug delivery because of its large surface area, rich blood supply, avoidance of the first-pass effect, relatively high permeability to many drugs and self-insertion. (C. 2005) However, this route has not been extensively exploited because of the broad inter-individual variability affecting some physiological factors like the pH and the presence of limited vaginal secretions that further vary depending on age and menstrual cycles. Although various possibilities are presently being investigated, there are only a limited number of vaginal dosage forms available. The currently available vaginal delivery systems, such as creams, foams, gels, irrigations, tablets, have some limitations, such as leakage, messiness and relatively low residence time owing to the self-cleaning action of the vaginal tract and often require multiple daily doses to ensure the desired therapeutic effect. (Knuth 1993) The vagina has unique features in terms of micro flora, pH and cyclic changes, and these factors must be considered during the development and evaluation of vaginal delivery systems. The pH of the vagina is maintained by lactobacilli which produce sufficient lactic acid to acidify vaginal secretions to pH 3.5-4.5. The pH changes with age, stages of menstrual cycle, infections and sexual arousal. The pH is important in terms of the design and the efficacy of drug delivery systems. The changes in hormone levels during the menstrual cycle lead to alterations in the thickness of the epithelial cell layer, the width of intercellular channels, pH and secretions. (Robinson 2006) The variations in enzyme activity (endopeptidase and amino peptidases) along with hormonal changes lead to the problems in achieving uniform drug absorption. Drug transport across the vaginal membrane may occur by a number of different mechanisms including transcellular and intercellular routes and vesicular or receptor-mediated transport mechanisms. The nature of the vaginal fluids can significantly affect drug absorption. The alternative vaginal delivery of various drugs such as bromocriptine, sildenafil, oxytocin, calcitonin, LHRH and analogues, insulin, human growth hormone, oxybutynin and steroids

Used in hormone replacement therapy or for contraception have been investigated.

The vaginal cavity has also been proven suitable for the delivery of locally acting drugs such as antibacterial and antiprotozoal, antifungal, antiviral, spermicidal agents, prostaglandins and steroids. (Guler 2000) (Acartürk 1996) (C 2001)

The traditional commercial preparations, such as creams, foams, gels, irrigations and tablets are known to reside in the vaginal cavity for a relatively short period of time owing to the self-cleaning action of the vaginal tract, and often require multiple daily doses to ensure the desired therapeutic effect. Many different approaches have been tested to develop novel vaginal drug delivery systems that can meet both the clinical and the patients' requirements. Considerable attention has been focused on the development of controlled delivery systems providing a long-term therapeutic concentration of drugs following a single dose. The composition of vaginal dosage forms will be the focus of interest in the future. Novel forms are liposomes, vaginal rings, cubic gels and formulations based on polystyrene and silicone elastomers. One interesting group of auxiliary agents is the mucoadhesive polymers, which are the basis of newly designed systems. Bioadhesion may be defined as the state in which two materials, at least one of which is biologic in nature, are held together for extended periods of time by interfacial forces. If this attachment is due to a mucus coating, the phenomenon is sometimes referred to as mucoadhesion (R. 2011).

Mucoadhesion is the interaction between a synthetic or natural polymer and a mucin surface, leading to a net attraction. The vaginal route appears to be highly appropriate for bioadhesive drug delivery systems in order to retain drugs for treating largely local conditions, or for use in contraception. Particularly, protection against sexually transmitted diseases, such as HIV is critical. To prolong the residence time in

the vaginal cavity, bioadhesive therapeutic systems have been developed in the form of semi-solid and solid dosage forms. The main advantages of the bioadhesive systems over the existing solid and semi-solid preparations are as follows:
-Low production costs,
-Avoidance of aqueous or organic solvents,
-Ease of self-administration with no need to use Applicators,
-Gel-like consistency in the activated state,
-Avoidance of local irritation phenomena,
-Rapid bioadhesion, prolonged residence time in the vaginal cavity even in absence of physiological secretions associated with a controlled drug delivery, extended dosing interval,
-improved chemical and physical stability.

Mucoadhesion is usually obtained by using both synthetic and natural bioadhesive polymers. The most commonly used mucoadhesive polymers that are capable of forming hydrogels are synthetic polyacrylates, polycarbophil, chitosan, cellulose derivatives (hydroxyethylcellulose, hydroxypropylcellulose, and hydroxypropylmethylcellulose), hyaluronic acid derivatives, pectin, tragacanth, carrageenan and sodium alginate. Recently, thyolated polymers have been explored as new mucoadhesive molecules. The bioadhesive properties are ensured by polyacrylic acid-based polymers, known as Carbomers. A number of different commercially available Carbomer grades may be used. They vary in their molecular weight, degree of cross linking structure or residual components. Among the polyacrylic acid polymers, polycarbophil, a polyacrylic acid cross-linked with divinylglycol, is preferred. This water insoluble polymer has an apparent pKa of approximately 4.5 and picks up 60 - 100 times its weight in water.

VAGINAL ANATOMY AND PHYSIOLOGY:

The human vagina is a tubular, fibro muscular organ that extends from the cervix of the uterus to the vaginal vestibule measured in a length of approximately 9 cm. The vaginal blood supply comes from the internal iliac arteries branching into a complex network of arteries and veins surrounding the vaginal wall. Blood leaving the vagina enters the peripheral circulation via the internal iliac veins, thus bypassing the liver. (al 1996) (Hussain 2005)

Histologically vagina consists of three distinct layers: an epithelial layer, a middle muscular layer and an outer fibrous layer. The epithelial layer is classified as a noncornified, stratified squamous epithelium. This layer is composed of lamina propria and an epithelial cell layer which contain particulate glycogen (Figure 1). The normal thickness of the vaginal epithelium is approximately 200 µm. It is usually considered to be a mucosal surface, although it has no goblet cells and lacks the direct release of mucins. The surface of vagina has a number of folds or also called rugae, which increase the surface area of the vaginal wall.

Despite of the absence of any glands, the vagina produces a large amount of fluid. This fluid is a mixture of several components and includes leukocytes, inorganic and organic salts, mucins, proteins, carbohydrates, urea and fatty acid. Like the thickness of the epithelial layer, the amount and composition of the vaginal fluid change upon the hormonal activity.

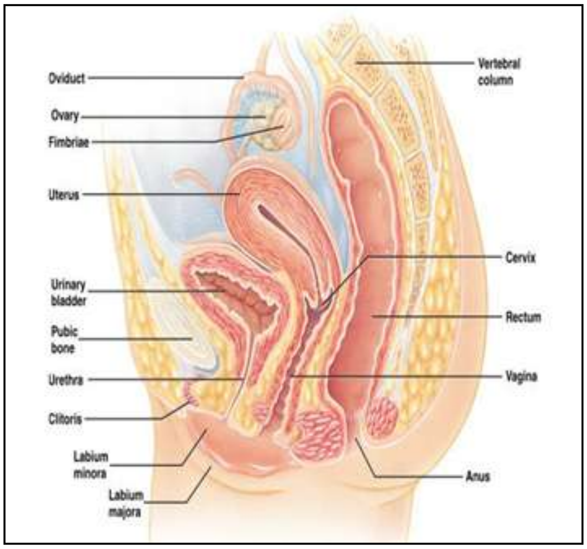

Figure 1: Lateral view of the female pelvis showing the absorption route to the systemic circulation.

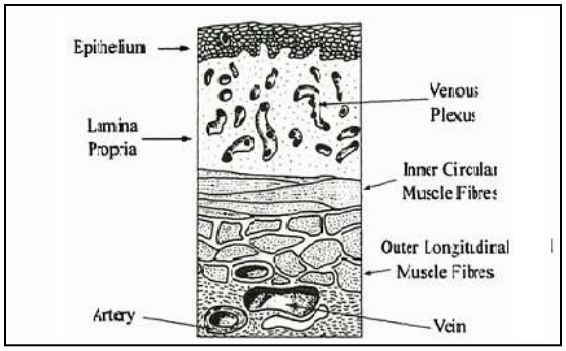
Epithelium
Lamina Propria
Venous Plexus
Inner Circular Muscle Fibres
Outer Longitudinal Muscle Fibres
Artery
Vein

Figure 2: Schematic illustration of vaginal wall

The lactobacilli bacteria are an important component of the vaginal micro flora. This bacteria converts glycogen from exfoliated epithelial cells into lactic acid, and as a result, maintains the pH around 4-5, with lowest being around the cervix. Body fluids such as menstrual blood, cervical and uterine secretions will all act as alkalizing agents and increase the vaginal pH.

VAGINAL ENVIRONMENT:

The volume, viscosity and pH of vaginal fluid as well as the thickness and porosity of epithelial layer may have either negative or positive impact on vaginal drug absorption. Therefore it is crucial to understand the conditions that might influence these parameters. It is noteworthy that the histology and physiology of the vagina may vary with age and with the menstrual cycle. Post-menopausal women experience important changes in the vaginal physiology. (Hussain 2005) These changes manifest as decline in estrogen production during the pre-menopause and ongoing menopause. This leads to a permanent decrease in the vaginal glycogen content, and consequently thinning of the vaginal epithelium. Furthermore, elevation of vaginal pH to 6.0–7.5, causes a decrease in the quantity of vaginal secretion. It was estimated that the vaginal secretion produced by postmenopausal women is reduced by 50% compared to that produced by women of reproductive age. Menstruation is another physiological factor associated with hormonal events. (d. N. al 2006) The epithelial layer changes in thickness by approximately 200-300 µm as estrogen levels change throughout the menstrual cycle. Changes in the vaginal pH and viscosity during the menstrual cycle are results of these changes in vaginal\ histology and physiology. The vaginal pH tends to be lowest at ovulation when estrogen levels reach a peak and both glycogen accumulation and epithelial desquamation at its maximum. These physiological cyclic variations will be affected by the use of oral contraceptives. (Hussain 2005)

Progestin-containing formulations are associated with the production of viscous mucus throughout the treatment cycle. (Washington 2001) The vaginal pH can be altered by pathological conditions such as infections and inflammations. Diseases such as candidal vaginitis may lower the pH to below 4.5, whereas the inflammatory vaginitis could elevate the pH to over 6.0. (C. 2005)

Age-related changes	**pH**	**Length of vagina(cm)**	**Width of vagina(cm)**
Prepuberty	7	4.5-6	1-1.5
Adult premenopause	4-5	7-8	2
Post menopause	4-7	4.5-6	1-1.5

Table 1: Influence of Age on Variation of pH, Length and Width of Human Vagina.

IDEALITY OF VAGINAL DRUG DELIVERY SYSTEM:

1) Component should melt at vaginal temperature i.e. at 36 °C.

2) Intra-vaginal drug delivery device should be non-toxic and non-irritating. (W. A. al 1996)

3) It should not have any meta-stable form.

4) The preparation should have high water number.

5) The preparation should have wetting and emulsifying properties.

6) The preparation should be non-sensitized on vaginal pH (i.e. 3.5-4.9).

7) It should be stable on storage.

8) The preparation should have small interval between melting and solidification point. (Jain 1997)

9 The preparation should have proper viscosity, so avoid the leakage of preparation from vagina (in case of semisolid dosage form).) (Rich*ardson 1992*)

10) The preparation should have proper bioadhesive/ mucoadhesive properties, so increase the contact time between the membrane and preparation.

ADVANTAGES OF VAGINAL DRUG DELIVERY SYSTEMS:

This route is the most preferred and targeted goal of new drugs and dosage forms, vaginal administration can be used as an alternative route in certain cases of therapeutic importance:

1) In cases of nausea and vomiting, the act of taking medication orally may induce emesis so that drug is vomiting before it is absorbed.

2) Irritation to the stomach and small intestine associated with certain drugs can be avoided.

3) Hepatic first pass elimination of high clearance drugs may be avoided partially (Ozer 2007).

4) Contact with digestive fluid is avoided, thereby preventing enzymatic degradation of some drugs.

5) Drug delivery can be stopped by removing the dosage form e.g. vaginal rings.

6) Drugs which traditionally are only given parentally may be administered vaginally either as such or in combination with absorption-promoting additives.

7) Rapid drug absorption and quick onset of action can be achieved.

8) Convenient for the patients, especially for those on long term therapy, when compared with parenteral medication.

9) The vaginal bioavailability of smaller drug molecules is good.

10) The bioavailability of larger drug molecules such as proteins and peptides can be improved by means of absorption enhancer or other approach.

11) Self-medication is possible.

12) Easy accessibility and good blood supply.

13) Relatively low enzymatic activity.

LIMITATIONS OF VAGINAL DRUG DELIVERY SYSTEMS:

1) Some of the drugs are sensitive at the vaginal pH.

2) Local irritation of some drugs.

3) Influence of sexual intercourses.

4) Gender specificity.

5) Personal hygiene.

6) Sometimes leakage of drugs from vagina and wetting of under garments.

7) Menstrual cycle associated vaginal changes.

8) Coitus interference.

9) Less preferable in terms of convenience.

10) Permeability of the vagina is strongly influenced by the estrogen concentration, which can influence the pharmacokinetics of drugs designed for systemic action. (Deshpande 1992)

FACTORS AFFECTING DRUG DELIVERY THROUGH VAGINAL MUCOSA:

Absorption:

A good understanding of the various factors that can influence drug absorption from the vaginal cavity is very important in designing both the formulation and the device used for intra-vaginal administration. There are several factors ranging from physiological conditions, physic-chemical properties of the drug, factors related to the administration device.

1) Physiological Factors:

Factors related to the vaginal physiology include pH of vagina (3.5 to 4.9), effect of the estrus cycle on the permeability of the vaginal mucosa, thickness of vaginal epithelium, vaginal fluid volume, chemical composition of fluid, pH, viscosity and surface tension and the pressure exerted on the dosage form by the rectal wall, play a vital role in vaginal drug absorption and sexual arousal, mucociliary clearance (MCC), vaginal obstruction, etc. which affect either the mucus or ciliary heating and vaginal blood flow.

2) Physico-chemical Factors:
Factors related to the dosage forms are physicochemical characteristics of the active ingredients; pH and mucosal irritancy; osmolarity; viscosity (solution, gels) and density (powder, tablet) to the formulation; concentration and volume administration; and type of dosage forms; particle size of the molecule of drug, hydrophilicity or lipophilicity of drug molecule, molecular weight of drug molecule, chemical nature, ionization surface charge, etc.

3) Factors Related to the Administration Device:

It includes the viscosity of the semi-solid dosage forms (creams, gels, etc), size of the solid dosage form (vaginal tablet, etc) adhesive property of the dosage form.

Improvement of Vaginal Absorption:

The low vaginal absorption can be the result of poor membrane permeability due to molecular size, lack of lipophilicity (e.g. steroidal drugs) fluid volume, estrus cycle and pH of vagina. To overcome this problem, most reported studies have investigated penetration enhancers to facilitate the transport of these molecules and improve the bioavailability. In general, enhancers improve the absorption of these molecules by one or several combined mechanisms:

1) By increasing intracellular transport or use of penetrating agents e.g. PEG.

2) By increasing the contact time between the dosage form and the vaginal membrane by using muco-adhesive polymers e.g. Carbopol 934, 940, etc and pormulation of gel and by increasing viscosity of formulation.

3) By increasing vaginal blood flow, thereby raising the concentration gradient across the vaginal mucosa.

4) By the use of bio-adhesive preparations have been developed as a new type of controlled release form for the treatment of both topical and systemic diseases. The greatest advantage of such dosage forms is the possibility of maintained them in the vagina for extended periods of time including day hours and night, thereby enabling lower dosing frequencies. Among the polymers polyacrylic acid (PAA) and HPMC (Hydroxypropyl methyl cellulose) are the ideal excipients in bio-adhesive vaginal preparations due to their high bio-adhesive strength.

5) By the use of chelating agents as a penetration enhancers in vaginal formulations. Vaginal administration of the protein leuprolide is much more effective when enhancers, such as carboxylic acids with chelating ability are co-administered.2

6) By the use of pro-drugs enhances drug permeability through modification of the hydrophilicity or lipophilicity of the drug. The method includes modification of chemical structure of the drug molecule, thus making it selective, site specific and a safe vaginal drug delivery system.

7) By the use of gel formulation is an extreme case of viscosity enhancement through the use of viscosity enhancers. So the dosing frequency can be decreased to once or twice a day. Example of vaginal gel is metronidazole gel. It is most popular vaginal gel. Mostly hydrogels are used for the intravaginal gel drug delivery system.

VAGINAL PHARMACOKINETICS:

If the absorption, distribution, and elimination of a drug molecule after its release from a vaginal drug delivery device in the vaginal lumen follow the pharmacokinetic sequences,

Drug in tissue
compartment (Ct)

Kct ↑↓ Ktc

Drug in vaginal
delivery device
↓release

Drug in vaginal fluid(Cv) $\rightarrow K_a \rightarrow\rightarrow$ Drug in tissue
compartment
(Cc)

↓ Ke

Elimination

The rate of change in drug concentration in the central compartment can be expressed by:

$$d\,(Cc)/dt = Ka.Cv + Ktc.Ct - Kct.Cc - Ke.Cc$$

$$d\,(Cc)/dt = Ka.Cv + Ktc.Ct - (Kct + Ke).Cc \ \ldots\ (1)$$

The rate of change in drug concentration in the
Body can be expressed by:

$$d\,(CB)/dt = Ka.Cv - Ke.CB \ \ldots\ldots\ \ldots\ldots\ (2)$$

Where
CV = Concentration of drug in vagina.
CB = Concentration of drug in body.

- *PERMEATION OF DRUG MOLECULE THROUGH VAGINAL MUCOSA:*

The permeability of drug through vaginal mucosa is effected estrus cycle, low lipophilicity of drug and the permeability of drug through the vaginal mucosa is dependent upon the drug concentration in the vaginal fluid means follow the first-order rate process.
The apparent permeability coefficient Papp for vaginal membrane permeability is defined by:

$$Papp = 1/\,(1/Paq + 1/\,Pv) \ \ldots\ldots\ (3)$$

Where

Paq = Permeability coefficient of the aqueous diffusion layer.
Pv = Permeability coefficient of the vaginal membrane.

Because the permeability coefficient of the vaginal membrane is depends upon the permeability coefficient of aqueous pore pathway (Pp) and lipoid pathway (PL), means

$$Pv = Pp + PL$$

So, eq. (3) will become

$$Papp = 1/\,\{1/\,Paq + 1/\,(Pp + PL)\} \ \ldots\ (4)$$

- *VAGINAL ABSORPTION:*

Much has been written in the literature concerning vaginal absorption. The first experimental studies using animals dates back to 1918. At that time, the histological characteristic of the vaginal wall was known to exist in three simple layers: the connective tissue, muscular, and the mucosa, collectively resembling the skin without the stratum corneum. Originally, the vagina was regarded as an organ impermeable to exogenous agents. Reports began to surface that indicated vaginal absorption of foreign materials as the cause of toxicity and even death in several cases. Using dogs and cats, vaginal absorptions of large varieties of compounds, including alkaloids, inorganic salts, esters, and antiseptics, were demonstrated. Later work showed the vaginal absorption of compounds such as hydrocyanic acid, pilocarpine, atropine, and insulin in dogs and cats. Using rabbits, cats, and dogs, the vaginal absorption of quinine bisulfate and oxyquinoline sulfate was set forth, intending to emphasize the care and consideration required of physicians with regards to the local applications of medication to the vaginal mucosa. A unidirectional transmission of agents was proposed to occur from the vagina to the blood, with no transmission in the reverse direction. An extensive review of the literature documented the absorption of carbohydrates, fats, and proteins. Glucose was absorbed and rapidly oxidized. One of the first proteins demonstrated to be absorbed vaginally was peanut protein. The vaginal permeation of spermatozoa and bacterial antigens also has been shown. Bacterial antigens play an important role in triggering the local immunological mechanisms involved in protecting the area against infection. Other classes of compounds include steroids (e.g., estrogens, progesterone, and testosterone), prostaglandins, antimicrobials, non-oxynol-9, and methadone. The vaginal delivery of estrogen and progesterone has been well documented over the years and used clinically in dosage forms, such as vaginal creams and suppositories. Vaginal absorption of drugs is dependent upon such physicochemical properties as molecular weight, lipophillicity, ionization, molecular size, chemical nature, and local action, as well as the thickness of the vaginal wall as affected by the ovarian cycle or pregnancy. Other factors include changes in the vaginal epithelium and pH with menopause. Prior to absorption, drugs must be in solution. The fluid present in the vagina can help to dissolve drugs, but the cervical mucus secretion also can present a barrier and remove a drug from the site when abundant. Dosage forms can undergo different absorption due to the differing dissolution patterns in vaginal fluid. Products such as creams, inserts, and tablets remain for different periods of time in the vagina. A comparison of vaginal inserts versus creams showed that creams have a longer contact time in the vagina. Drug transport across the vaginal membrane may occur by a number of different mechanisms:

a) Diffusion through the cell due to a concentration gradient (transcellular route),

b) Vesicular or receptor-mediated transport mechanism, or

c) Diffusion between cells through the tight junctions (intercellular route).

In some cases, drugs given by the vaginal route have a higher bioavailability compared to the oral route. This is because the drug enters immediately into the systemic circulation without passing the metabolizing liver. The vaginal wall is very well suited for the absorption of drugs for systemic use, since it contains a vast network of blood vessels.

CLASSIFICATION OF MUCOADHESIVE VAGINAL DRUG DELIVERY SYSTEMS:

The vaginal drug delivery systems are categorized as follows:

1) Mucoadhesive Vaginal gels
2) Mucoadhesive Vaginal tablets
3) Mucoadhesive Vaginal films
4) Emulsion type vaginal drug delivery systems
5) Pessaries
6) Creams, Foams and Jellies
7) Vaginal rings

1) Mucoadhesive Vaginal Gels:

The most widely used mucoadhesive vaginal drug delivery systems are gels. (d. N. al 2006) In particular, for drugs designed for gynaecological use, a bioadhesive gel able to ensure prolonged contact between the active ingredient and the vaginal mucosa, and gradual release of that ingredient over time, provides the ideal solution in terms of efficacy and compliance by patients. Among vaginal formulations, gels are easy to manufacture, comfortable, and have the ability to spread onto the surface of mucous and to achieve an intimate contact with vaginal mucosa. Moreover, because of their high water content and their rheological properties, they present the further advantage of a hydrating and lubricating action, which is particularly useful in pathological situations characterized by dryness of the vaginal mucosa. The employment of mucoadhesive polymers can improve the time of contact with the mucosa, delaying the loss of the formulation and prolonging the effect.

a) Gels for the Moisturization of Vagina:

Vaginal dryness and pain is a problem experienced by women due to the decreasing hormone levels at the onset of menopause. (C. 2005) There are a number of patents for the moisturization of the vagina in the case of vaginal dryness. One of the first marketed products is Replens® gel which consists of 1-3 % polycarbophil. It is retained in the vaginal cavity for 3-4 days. Robinson JR described the bioadhesive polymers in a vaginal tissue moisturizing composition. (Mauck 2008) The composition utilized in these inventions include water, a bioadhesive polymer as the moisturizing agent and a gel-like consistency-enhancing agent. The bioadhesive is a waterswellable, but water-insoluble, fibrous, cross-linked carboxy functional polymer. The consistency-enhancing agent is a water-soluble or water-dispersible anionic or non-ionic polymer. It is polyacrylic acid cross-linked with polyallyl sucrose containing an average of at least three allyl groups per molecule. The composition is contacted with the vaginal mucous membrane for a time period sufficient to moisturize the contacted area. (Hussain, The vagina as a route for systemic drug delivery 2005)

In the patent, the method of moisturizing the dry vagina was the following:

A moisturizing composition containing dry polycarbophil sized to pass through a 400 mesh sieve screen and whose average longest dimensions are less than about 20 microns, Carbopol.RTM. 934, a hydrogenated palm oil glyceride dispersant, heavy mineral oil, glycerin U.S.P., methyl paraben and distilled, deionized water up to 1000 g was prepared by admixing with agitation as an emulsion. The pH value was adjusted to pH 2.4 with a solution of sodium citrate in HCl. About 4 g of the above composition are placed into a plunger-type applicator. The applicator and its contents were placed into the vagina of a post-menopausal woman presenting with dry vagina and vaginitis, and the plunger was depressed to expel the composition into the subject's vaginal cavity to thereby contact the vaginal mucosa. The composition so applied moisturized the mucosa and also provided lubrication for sexual intercourse. In this invention, bioadhesion was measured as a function of pH value and the maximum adhesion was observed at a pH value of about 5 to about 6.

A gel formulation for the local hormonotherapy of vaginal dryness was developed and disclosed in a world patent. The gel is intended for local treatment, essentially non systemic, of vaginal dryness, particularly in menopausal women. It is characterized by the synergic combination of a natural oestrogen selected amongst 17 betaestradiol and its salts and a biodegradable, hydrophilic lubricant and bioadhesive gel. The excipient comprises preferably carboxyvinylic acid, glycerin, a pH adjusting substance, a preservative adjuvant and an aqueous excipient. A mucoadhesive aqueous gel formulation for vaginal use was described in the patent of Giroux . In this invention the mucoadhesive gel comprises 1-15 weight% natural polysaccharides, 0.5-15% mucoadhesion promoter and water. This invention relates to a moisturizing aqueous gel for local use, especially for the hydration of the vaginal mucosa. In this invention, the chitins (nonionic), carragenates or alginates (anionic), guar type polysaccharides (cationic) or mixtures of these polysaccharides can be used. Among the moisturizing agents physiologically acceptable, salts of pyroglutamic acid may be suitable for the present invention.

b) Gels for the Treatment of Vaginal Infections:

Gels or related semisolid dosage forms are also important candidates as microbicide delivery vehicles. A vaginal gel can serve several functions:

1) Delivering microbicide molecules to vaginal fluids and tissues

2) Maintaining a reservoir of these molecules within a layer that can serve as a barrier to HIV migration from semen to tissue

3) Providing lubrication to diminish tissue damage during sexual activity.

A patent for a prolonged-release bioadhesive vaginal gel dosage form has been described by Durrani. The dosage form comprised a synergistic formulation of carrageen, acrylic acid containing polymers (carbophil), ultra low gelling temperature agarose and an effective amount of a therapeutic agent. The first release rate of the therapeutic agent from the composition is greater than the second rate. The therapeutic agent is released for up to about 24 hours. The polymer comprising acrylic acid is selected from the group consisting of copolymers of acrylic acid, polycarbophil, homopolymers of acrylic acid crosslinked with divinyl glycol, polyacrylic acid homopolymers, carbomers, Carbopol 974P-NF, Carbopol 971P-NF, ETD resins, and copolymers of acrylic acid and Cl0 to C30 alkyl acrylic acid. A method for making a bioadhesive, prolonged release drug composition consisted of the following steps:

(a) Dissolving appropriate amounts of soluble components comprising sodium chloride, methylparaben, acetate buffer, and, optionally, at least one therapeutic agent, in water to provide a first mixture.

(b) Dispersing appropriate amounts of gelling agents comprising ultra low gelling agarose and carrageenan in the first mixture to produce a second mixture, this is stirred for about one hour.

(c) Dispersing an appropriate amount of at least one polymer comprising acrylic acid in the second mixture producing a third mixture, which is stirred and heated to about 90oC, and which is further cooled and stirred at about 70oC; and

(d) Cooling the third mixture to room temperature and stirring it until uniform, and, optionally, adding at least one therapeutic agent. The therapeutic agent was selected from the group consisting of a spermicide, an antiviral, an antibacterial, an antifungal, an antimycotic, an antipruritic, an emollient, a humectant, an anti-inflammatory, an immunomodulator, a hormonal, an antineoplastic and an analgesic. Similary, semisolid mucoadhesive formulations for vaginal application with improved technical and organoloptic characteristics, which contain at least two bioadhesive gelling polymers and an active ingredient, useful in the prevention and/or treatment of various pathologies and disorders in human beings or animals, were described in a US patent. This semisolid mucoadhesive formulation contained: a first bioadhesive gelling polymer that is a polyacrylic acid crosslinked with divinyl glycol (Polycarbophil AA1) (0.1- 5% by weight of the semisolid mucoadhesive formulation); a second bioadhesive gelling polymer that is a polyacrylic acid crosslinked with allyl sucrose or allyl pentaerythritol (Carbopol 971P, Carbopol 940, Carbopol 941, Carbopol 980, and Carbopol 981) (0.1-5% by weight of the semisolid mucoadhesive formulation); a neutralizing agent in sufficient quantity to position the semisolid mucoadhesive formulation's pH between 2 and 6; a pharmacologically active agent; and water. The pharmacologically active agent is selected from a group consisting of hormones, antibacterials, antimycotics, antiprotozoals, anti-STD agents, spermicides, local anaesthetics, anti-inflammatories, labour inducers, and smooth muscle relaxants. The bioadhesion of the formulation was measured in both ovariectomized and non-ovariectomized rats and bioadhesion to the vaginal mucosa of ovariectomized rats is greater than that of the non-ovariectomized rats. Vaginal infections are a common problem among women. Bacterial vaginosis is the most common form of infectious vaginitis, accounting for 45% of symptomatic cases and estimated to be present in 15% of asymptomatic sexually active women. Microbicides can be formulated as creams, films or pessaries: gel formulations appear to be preferred among researchers. A bioadhesive aqueous composition patent assigned to Robinson and Bologna described the use of a bioadhesive aqueous composition to control the pH of the vagina to alleviate microorganism growth and feminine odour such as presented by bacterial vaginosis. The composition comprises water and an acidic polymer, specifically one wherein 80% of the monomers contain at least one carboxyl group[-COOH] and wherein the polymer is crosslinked so as to be water-swellable, but water-insoluble (polycarbophil). The composition of the present invention is additionally a bioadhesive agent providing for a long-lasting benefit and control of vaginal pH. The formulation further comprises an adjuvant selected from the group consisting of preservatives, lubricating oils, emulsifying agents, colouring agents, odour providing agents, and humectants. Another invention relates to a method for controlling the pH value in the vagina to suppress the growth of microbes and reduce female odour by means of a composition of bioadhesive and water. This composition contains water and acidic polymer, specifically the polymer whose 80% monomer contains at least one carboxyl (-COOH). This polymer is cross-linked and is swellable in water, but not dissolved in water, and this composition has bioadhesion for durable action.

Mucoadhesive antimicrobial complexes consist of a cross-linked polyacrylic acid having remarkable mucoadhesive properties, known as polycarbophil, and an imidazole or triazole derivative with antifungal or antiprotozool activity in its basic form, for use in the topical treatment of mucosal affections. Imidazole derivative is chosen from the group consisting of econazole, clotrimazole, metronidazole, tioconazole, fenticonazole, isoconazole, ketoconazole, sulconazole, bifonazole, omoconazole, azanidazole, butoconazole and oxiconazole. This formulation is produced by dissolving each of the two starting products in a common solvent or mixture of solvents, or in two different solvents compatible with each other, then joining together the two solutions in relative amounts and subsequently evaporating the solvent. Particularly preferred do formulations in gel in propylene glycol comprise an econazole polycarbophil or omoconazole-polycarbophil complex, with an excess of polycarbophil, together with pharmaceutically acceptable carrier and excipient substances, for use as sustained-release antifungals for vaginal administration. Another patent was for vaginal or transvaginal treatment of fungal, bacterial, viral or parasitic infections in human females. A device was developed comprising a pharmaceutical agent, a pharmaceutically acceptable non-toxic lipohilic or hydrophilic carrier, mucoadhesive agent and a penetration enhancer. An effective amount of a pharmaceutical agent selected from the group consisting of miconazole, terconazole, isoconazole, fenticonazole, fluconazole, nystatin, ketoconazole, clotrimazole, butoconazole, econazole, metronidazole, clindamycin, 5-fluoracil, acyclovir, AZT, famovir, penicillin, tetracycline, erythromycin is formulated as a vaginal suppository, bioadhesive tablet, bioadhesive microparticle, cream, lotion, foam, ointment, paste, solution, and gel incorporated into a vaginal device with a non-toxic pharmaceutically acceptable carrier. The composition comprises a lipophilic carrier (a semi-synthetic glyceride of saturated fatty acids of 8-18 carbon atoms) or hydrophilic carrier (polyethylene glycol of a molecular weight from 400 to 6000) and a mucoadhesive agent (alginate pectin or hydroxypropyl methylcellulose) for intravaginal delivery. A penetration enhancer or sorption promoter (nonionic surfactant, bile salt or ethoxyglycol) is added to this formulation for transvaginal delivery. The device is a tampon, tampon-like device, vaginal ring, vaginal pessarie, vaginal cup, vaginal tablet, vaginal suppository, vaginal sponge, vaginal bioadhesive tablet, vaginal bioadhesive microparticle, comprising pharmaceutical agent formulated as a cream, lotion, foam, ointment, solution or gel.

An interesting patent for treating or preventing vaginal infections has been described by Bologna and Levine. In this invention the pharmaceutical vaginal composition includes a synergistic mix of a bioadhesive, extended release formulation that decreases the pH and that contains peroxide in an amount sufficient to increase oxygen concentration without sterilising the vagina or substantially killing the normally-desired local vaginal flora. The synergistic mix releases peroxide over a period of at least 24 hours. The peroxide source is carbamide peroxide. The extended-release formulation includes a bioadhesive, waterswellable, water-insoluble, cross-linked polycarboxylic polymer (polycarbophil). Larsen described a similar gelled vaginal pharmaceutical composition containing a water-soluble bioadhesive polymer, a peroxide source and pH buffer. The pH of the composition is between about 3.0 and 6.0. The peroxide source of the formulation is hydrogen peroxide. The water-soluble polymer is an acrylic acid modified polymer. The composition contains a spermicide (nonoxynol-9) or a therapeutic agent which is selected from the group consisting of antibacterial, antiseptic, antibiotic, anti-inflammatory, antiparasitic, antiprotozoal, antiviral, antifungal agent and mixtures of these. The water-soluble polymer is an acrylic acid polymer and is used in amounts of about 1.0% to about 3.0% by weight of the formulation. Another formulation for topical treatment of mixed vaginal infections was described by Bortz *et al.* In this invention a pharmaceutical composition comprises an antibacterial agent (clindamycin) and an antifungal agent (butoconazole). The composition is adapted for application in a unit dose amount to a vulvovaginal surface and has at least one nonlipoidal internal phase and at least one lipoidal external phase that is bioadhesive to the vulvovaginal surface. The clindamycin phosphate and butoconazole nitrate are present in substantial part in the internal phase. A phospholipid can be used as the emulsifying agent. The composition is useful for administration to a vulvovaginal surface to treat a mixed bacterial vaginosis and vulvovaginal candidiasis infection. The release period of the formulation was about 3 hours to about 10 days. Palacin *et al.* developed a formulation for the treatment of vulvovaginal candidiasis. The invention relates to monodose mucoadhesive vaginal compositions of sertaconazole or a pharmaceutically acceptable salt for the treatment of vulvovaginal candidiasis. The lipophilic excipients are selected from glyceryl stearates and their derivatives; ketostearyl alcohols, polyoxyethylene glycol ethers of nalcohols, liquid paraffin, lecithin oil and glycerol are present in a total proportion of from 10 to 40%. The mucoadhesive excipients are selected from cellulose polymers, gelatin, colloidal anhydrous silica and polyacrylic acid polymers which are cross-linked with divinyl glycol and acrylic acid polymer cross-linked with sucrose or pentaerythritol allyl esters. The preservatives are selected from parabens, benzoic acid, sorbic acid, boric acid and the like.

c) Gels for Contraception:

A galenic form of vaginal application as a local Contraceptive and/or for protection against STD and/or AIDS was described by Meignant. This formulation consists of an external envelope containing gelatine and inner non-aqueous liquid or semi-liquid phase containing a dissolved active component of spermicide, a lipophilic agent compatible with the rubber for condoms, a water-dispersible agent, a bioadhesive agent and an agent for the gelatinization of the lipophilic agent. The formulation simultaneously has spermicidic and lubrication properties. This vaginal dosage form combines the advantages associated with a soft capsule with spermicidal, antiseptic and lubricating properties. It can be safely used in conjunction with condoms without altering their rubber latex composition. The bioadhesion agent is a bio-compatible polymer selected from: carboxyvinyl acids; carboxymethylcellulose; sodium carboxy-methylcellulose; methylcellulose; hydroxypropylcellulose; hydroxypropylmethylcellulose; agar-agar; aluminum silicate; carrageenates; and carob gum, whereas the spermicide agent is selected from benzalkonium chloride, benzethonium chloride, cetyl pyridinium chloride, methylbenzethonium chloride, tetra-decyltrimethyl ammonium bromide, benzalkoniu bromide, monylphenyl ethers, lauryl ethers, and octoxynols.

d) Formulations Based on Cellulose Derivatives and Chitosan:

In most of the formulations, acrylic acid polymers (Carbomer or polycarbophil) are used as a viscosity-controlling or bioadhesive agent. Chitosan and cellulose derivatives were also used in mucoadhesive formulations instead of the acrylic acid polymer. However, the problem of obtaining a bioadhesive formulation that presents all of the following advantages and properties remains substantially unsolved: release of drug for up to approximately 24 hours; absence of gelling/bioadhesive agents, characterised by the presence of acid groups, which are therefore sensitive to the ionic strength of the medium, and sometimes need to be neutralised with bases; the possibility of carrying drugs with different chemicophysical properties, in particular water-soluble drugs and lipophilic drugs which are substantially insoluble in water; reduction of the time and cost of the treatment. It has now been found that said objectives can be achieved by bioadhesive gel formulations that adhere to the mucous membranes, in particular the vaginal mucosa, comprising hydroxyethylcellulose as the only bioadhesive polymer. This gelling excipient has no acid groups and is therefore not dependent on the ionic strength of the medium; it also has a matrix effect, which allows particularly slow, gradual release of the active ingredient, for up to 24 hours. Cellulose derivatives, such as hydroxyethylcellulose, hydroxypropylcellulose and hydroxypropyl methylcellulose have also been used as mucoadhesive polymers. A bioadhesive gel based on hydroxyethycellulose was described in a US patent. This composition in the form of an aqueous bioadhesive gel adapted for the delivery of active ingredients comprised hydroxyethylcellulose (1-5 %) as the only gelling and bioadhesive agent, glycerol (5-25 %), diethylene glycol (5-50%) monoethyl ether, together with one surfactant, preservative (0.05-1%) and acidifier. An active constituent is selected from the group consisting of antifungals, antiseptics, antimicrobials, antibiotics, analgesics, local anaesthetics, antihistamines, anti-inflammatory agents, contraceptives, hormones, and combinations of these. Tzachev and Popov described a liquid mucoadhesive composition to be applied to the mucosal epithelia with prolonged and improved coating and protection effect. The liquid pharmaceutical composition contained xylomethazoline HCl (0.1-10%), hydroxypropyl methylcellulose (0.01-10%) disodium ethylenediamine tetraacetate (0.01-10 %) and phosphate buffer. The composition according to the invention can be applied easily and has a prolonged contact to the mucosal surface.

Caramella *et al* used chitosan for the preparation of the pharmaceutical composition for vaginal administration. The new pharmaceutical composition is biodegradable and biocompatible with the vaginal cavity, comprising a gel of chitosan and lactic acid for the treatment of various types of bacterial vaginosis and for the restoring (recolonising) of the physiological flora of lactobacilli; this composition releases lactic acid gradually at a constant rate over a prolonged period of time controlled release, an acid which is physiologically present on the vaginal mucosa, with improved mucoadhesive properties and avoiding undesired contraceptive effects. In particular the weight ratio chitosan: lactic acid is between 1:1 and 2:1, and is preferably 1.8:1. In particular the gel has a pH between 3 and 5, preferably between 3.5 and 4.5. The gel, characterising the composition to which the present invention refers, has a chitosan concentration of between 1.5% and 5% of the weight, more preferably between 2% and 4% of the weight. A vaginal lubricant comprising high molecular weight hyaluronic acid combined with low molecular weight hyaluronic acid and/or chitosan which has improved stability has been developed by Zhao and Burd. This formulation has been applied for the maintaining and/or promoting sperm motility. The addition of chitosan helps to maintain the viscoelasticity of formulations.

e) Formulations Based on Thermosensitive Polymers:

In recent years, vaginal application of thermosensitive gels which presents mucoadhesive behaviour with mixtures of poloxamers and polycarbophil or cellulose derivatives has been gained more importance. Auzerie developed a vaginal formulation in the form of a gel provided for the treatment of mucosa with an active principle. The formulation includes thermoreversible gel, bioadhesive product and active principle in solution or suspension. The gel composition for the treatment of mucosa, particularly vaginal mucosa with an active principle, comprised in combination: a poloxamer Lutrol F 127, a bioadhesive product Carbopol 5984, and an active principle in solution or in suspension such as an antifungal, an antiseptic, an antibiotic, a contraceptive or a combination of two or more of these. A cellulosic derivative (hydroxypropyl methylcellulose) can be added to the composition. The formulation comprises at least one preservative and neutralizer. The preservative is selected from sodium methyl parahydrobenzoate or sodium propyl parahydrobenzoate, whereas the neutralizer is sodium hydroxide. The production of the composition is carried out in the following steps:

premixture 1; addition of a portion of the excipient to the neutralizer, premixture 2; dispersion of the active principle in a volume of excipient at 30 to 35 ˙C, and mechanical mixture of the thermoreversible gel, of the bioadhesive product and of premixtures 1 and 2. Despite the numerous vaginal gel formulations patented relatively few gel preparations are commercially available.

2) Mucoadhesive Vaginal Tablets:

Mucoadhesive polymers such as polycarbophil, cellulose ethers, chitosan and polyvinylpyrrolidine were used for the preparation of tablet formula. (Deim 2008)

The manufacturing process of vaginal bioadhesive controlled release matrix tablets consists of the preparation of a matrix mixture comprising the pharmaceutically acceptable excipients. The release mechanism is based on drug diffusion through the swollen polymers and progressive erosion /dissolution of the gel matrix. The controlled-release properties of the vaginal tablets may be modified by the presence in the dosage form of soluble and insoluble fillers and by their weight ratio. (Karasulu 2004) The insoluble excipients can be selected from the group of microcrystalline cellulose, calcium phosphate tribasic, dibasic calcium phosphate, calcium sulphate and dicalcium phosphate. Either anhydrous or hydrated dicalcium phosphate is preferred. The soluble excipients can be selected from the group of lactose, sorbitol, xylitol, mannitol, amylose, dextrose, fumaric acid, citric acid, tartaric acid, lactic acid, malic acid, ascorbic acid, succinic acid, polyethylene glycols of various molecular weight, soluble hydroxyalkylcelluloses, polyvinylpyrrolidones, gelatins, sodium carbonate and sodium bicarbonate. A hydrophilic matrix patent assigned to De Luigi and Mailland described a hydrophilic matrix containing a polyacrylic acid derivative (0.5-40%), a cellulose ether (30- 90%) and a disintegrant for the treatment of vaginal disorders such as vulvovaginal candiasis, bacterial vaginosis or trichomoniasis. The polyacrylic acid derivative is polyacrylic acid cross-linked with polyalkenyl ethers of sugars or polyalcohols (carbomer) or cross-linked with divinylglycol (polycarbophil). Cellulose ether is selected from hydroxyalkylcellulose (hydroxypropylmethylcellulose, hydroxylpropylcellulose and hydroxyethylcellulose), alkylcellulose (methylcellulose and ethylcellulose), carboxy-methylcellulose or a salt of these. Disintegrant is selected from modified celluloses such as cross-linked sodium carboxymethylcellulose, cross-linked polyvinylpyrrolidone such as cros-povidone, natural starches, such as maize starch, potato starch, directly compressible starches, modified starches, such as carboxymethylstarches and sodium starch glycolate, starch derivatives such as amylose, alginic acid and sodium alginate, microcrystalline cellulose. (Perioli 2009) Conte and Maggi described an antiviral pharmaceutical composition for vaginal administration. For this purpose bioadhesive, biocompatible sustained-release tablets were prepared. The hydrophilic bi-layered tablets are slowly erodible and/or disagregable. The first layer is able to release immediately the drug, and the second layer provides the sustained release of the drug by means of bioadhesive polymers. For the preparation of the compositions, the antiviral drug is selected from: acycloguanosine (acyclovir) or its salts or derivatives, trifluridine, bromovinyldeoxyuridine, desciclovir, enviroxime, ganciclovir, idoxuridine, inosine pranobex, interferons, rimantadine hydrochloride, ribavirine, vidarabine and derivatives, zidovudine or azidothymidine. Biocompatible bioadhesive polymers are selected from gelatine, xantanes, scleroglucane, collagene, pectin and amylopectine, dextranes, hyaluronic or polygalactouronic acid, alginic acid, alginates, polyvinylpyrrolidone, polyvinylalcohol, polyethylenglycols, polypropylenglycols and copolymers, polymethylvinylether maleic anhydride copolymer and derivatives, polyacrylic and methacrylic acid derivatives, carboxyvinylpolymers, cellulose derivatives, methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose and its salts in this patent.

Mucoadhesive tablets containing cross-linked poly (acrylic acid) as a polycarboxylated polymer were developed for the mucosal delivery of benzocaine. The bioadhesive composition comprises a mixture of a polysaccharide and a polycarboxylated polymer such as cross-linked poly (acrylic acid) (15-25%). To produce the bioadhesive system, a solution comprising water and a polymer mixture wherein the polymer mixture comprises one synthetic polycarboxylated polymer cross-linked poly(acrylic acid) component and one polysaccharide component(starch) is prepared and then dried to form a solid. The solid is compressed to form a tablet. A bioadhesive tablet for vaginal administration was described by Gilis. This invention is concerned with bioadhesive pharmaceutical compositions comprising a pharmaceutically effective amount of an active ingredient (80-98.8% w/w), pre-gelatinized starch and hydrophilic matrix forming polymer (1-10%). A hydrophilic matrix forming polymer is selected from the group consisting of polyacrylic acid, carbomer, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, sodium Carboxymethylcellulose, polyvinyl alcohol and a mixture of these. A dry process is used for preparing the tablets comprising the steps of: mixing the pharmaceutically active ingredient, the pre-gelatinized starch and the hydrophilic matrix forming polymer in the dry state until homogenous; compacting the thus obtained mixture into a sheet; breaking the sheet into a granulate; blending the granulate with the lubricant and optionally a glidant; and compressing the blend into tablets.

A rapidly dispersible vaginal tablet that provides a bioadhesive gel has been described in a patent assigned to Stone *et al.* The tablet contains a microbicide such as cellulose acetate 1, 2 benzenedicarboxylate (CAP) (0.01-500 mg), mannitol powder (100-500mg), microcrystalline cellulose (50-300mg), hydroxypropyl methylcellulose (10-80 mg), glycerol (50-250mg) and a preservative (2-4mg). The tablet which includes CAP as the vaginal medication is vaginally administered before coitus in methods for preventing the sexual transmission of HIV-1, HIV-2, herpes virus or an infection caused by *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Haemophilus ducreyi* or *Treponema pallidum*. The tablet which inludes CAP as the vaginal medication is vaginally administered to prevent or treat bacterial vaginosis. Sen *et al.* describes a sustained release, mucoadhesive vaginal pharmaceutical composition. This solid dosage form contains an effective amount of a pharmaceutical ingredient.

Mucoadhesive properties and capable of providing a sustained release of the active pharmaceutical ingredient, the hydrophilic matrix comprising a hydrophilic polymer having an average molecular weight of about 100.000. The active pharmaceutical ingredient is selected from the group consisting of antifungal agents, prostaglandins, hormones, estrogens, pharmaceutically acceptable salts or esters, isomers, derivatives and combinations of these. The hydrophilic polymer is a polyalkylene oxide and the polyalkylene oxide is a polyethylene oxide. In the patent of Lemarchand [89] a mucosal bioadhesive slow release carrier comprising an active principle, which can release the active principal for duration of longer than 20 hours, was described. This bioadhesive carrier contains at least one bioadhesive natural protein of vegetal origin pea proteins, natural soy proteins, natural potato proteins, natural wheat proteins, glialdin proteins and mixtures of these (10 to 40% by weight). The active principle is selected from: an antiviral, an analgesic, an anaesthetic, an antalgic, an antiinflammatory,an antibiotic, an antiseptic, an antiemetic and mixtures of these.

The bioadhesive natural protein of vegetal origin is selected from the group of natural hydroxypropyl methylcellulose, gelatin, alginic acid, alginic acid sodium salt, pectin, collagen, poloxamer, carbopol, microcrystalline cellulose, polyacrylic acid, polyethylene glycol and polypropylene glycol. The film has a controllable rate of gelling, swelling and degradation and is preformed into a device or is applied as a coating to the surface of a more complex drug delivery system. pH-Responsive film for intravaginal delivery of a beneficial agent has been prepared for the intravaginal administration of prophylactic and therapeutic agents. The invention provides a pH-responsive, biocompatible for intravaginal administration of a beneficial agent, comprising a biocompatible, hydrophilic polymer that is positively charged at a first pH and assumes an electronically neutral form at a higher pH; an effective amount of beneficial agent and optionally at least one film-forming binder. The responsive film may also include other additives such as plasticizers, sustained-release polymers, antioxidants and antimicrobial agents. In another embodiment, the pH-responsive film of the present invention comprises a

3) Mucoadhesive Vaginal Films:

Another mucoadhesive solid dosage form isvaginal film. Repka *et al.* described a bioadhesive hot-melt extruded film for topical and mucosal adhesion applications. The film is made from a precursor composition containing a water-soluble or water-swellable thermoplastic polymer, preferably HPC and/or PEO and a bioadhesive polymer. The film can also contain a therapeutic agent, preservative, buffering agent, antioxidant, super-disintegrant or absorbent, flavorant, colorant, water-insoluble polymer, organic acid, surfactant, film modifier and/or cross-linking agent. A mucoadhesive film formulation which is suitable for delivery of therapeutic agents to vaginal mucosa has been developed. Film composition for delivery of pharmacologically effective agents topically to vaginal mucosa comprises polymer which is hydrophilic, hydrophobic or amixture of both. The polymer is selected from the group consisting of laminated composition of a) a bioadhesive layer that serves to affix the film to a mucosal surface within polymer. This film formulation can be used for contraception, treatment and/or prevention of viral infections, treatment of vaginal infections, relief of vaginal itch, vaginal cleansing and enhancement of vaginal lubrication. Wilson *et al* described a coated vaginal device for vaginal delivery of therapeutically effective and/or healthpromoting agents. The vaginal device is partly or completely coated in or combined with a film. The coating of the device comprises a mucoadhesive composition comprising a mucoadhesive agent, water-insoluble additive, surfactant, penetration enhancer, a therapeutic and/or health-promoting agent. The therapeutic agent selected from the group consisting of an antimicrobial, vasodilator, nonsteroidal anti-inflammatory (NSAI), prostaglandin inhibitor, COX-1 inhibitor, COX-2 inhibitor, local anesthetic, calcium channel antagonist, potassium channel blocker, [beta]-adrenergic agonist, bisphosphonate, leukotriene blocker, smooth muscle inhibitor, peptide, protein, dyskinetic muscle contraction inhibitor and anti-HIV agent and a combination of them whereas the health-promoting agent selected from the group consisting of a botanical, probiotic microorganism, vitamin, antioxidant, anti-pruritic additive and synergistic additive agent and a combination of them. Hydroxypropyl methylcellulose, sodium alginate, polyethylene glycol, carbopol, chitosan and propylene glycol alginate have been used as the water-soluble polymers. The water-insoluble polymer selected from the group consisting of microcrystalline cellulose, cellulose fibers, polyethylene and polypropylene whereas inorganic or organic pH buffering agent selected from the group consisting of sodium bicarbonate, sodium carbonate, sodium phosphate, citric acid, sodium citrate, lactic acid, acetic acid, sodium acetate and a combination of them. A pharmaceutical composition for the controlled release of medicinal drugs, which has the property of adhering to biologic tissues has been prepared. The characteristic features of the composition are a plurality of small-size units capable of ensuring a gradual release of the active ingredient they contain the units being coated with a biodhesive polymer layer. Paris described a film formulation. The invention relates to novel viscous liquid compositions the vagina b) at least one reservoir layer comprising one beneficial agent and a biocompatible hydrophilic for producing pasty forms having a prolonged action and/or release for local applications. These compositions are characterized in that the long-lasting action and/or the prolonged release of the active substance is obtained by the *in situ* formation of a matrix film having an increased bioadhesive power and being more or less viscous and biodegradable. The invention also relates to a viscous liquid composition with an increased bioadhesive power for a local application in pasty form with a prolonged release of an active substance. It is characterized in that it contains at least one matrix agent, a medium for hydrating the matrix agent, and at least one active substance. Fatty acid esters have been used as bioadhesive substances. For this purpose, fatty acid esters are selected from the group consisting of fatty acid esters of polyhydric alcohols, fatty acid esters of hydroxycarboxylic acids, fatty acid esters of glycerylphosphate derivatives, fatty acid esters of glycerylsulfate derivative and mixtures of these. Excellent bioadhesive properties have been observed for fatty acid esters, such as glycerylmonooleate, glyceryl monolinoleate, or glyceryl monolinolenate.

4) Emulsion Type Mucoadhesive Vaginal Systems:

Emulsion type bioadhesive drug delivery systems offer different approach for vaginal administration. Perioli *et al.* designed a new mucoadhesive semisolid dosage forms (emulgels o/w) containing mucoadhesive polymers (HEC and NaCMC) for vaginal delivery of benzydamine. For this purpose simple gels (HEC 5 % and NaCMC 3 % and 4 %) were formulated and then added as outer water phase of an oil-in-water emulsion named “cetomacragol-based cream”. The most suitable emulgels for vaginal application were those containing NaCMC (3 %). This formulation showed the best *in vitro* and ex vivo performances A submicron emulsion formulation for delivery of peptides has been prepared by Friedman *et al.* This invention provides compositions and methods of administering peptides in an emulsion comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide and an aqueous continuous phase, which promotes absorption of the bioactive peptide through mucosal surfaces by achieving mucoadhesion of the emulsion particles.

Putteman *et al.*prepared mucoadhesive emulsion containing cyclodextrin. The composition comprised a drug selected from the group consisting of antibacterial, antiviral, contraceptive, and antifungal agents, and a cyclodextrin or a derivative in an amount from 10% to 70% by weight based on the total weight of the composition. A mucoadhesive emulsion composition comprised by weight based on the total weight of the composition 0.5 to 3% itraconazole; 30 to 70% cyclodextrin; 0.1 to 1% thickening agent; 1 to 5% emulsifier; 1 to 4% stabilizing agent; buffer, acid or base to maintain the pH of the composition between 1 and 3; 0.5 to 50% of a dermatologically acceptable oil; and water. The drug was selected from the group consisting of antibacterial, antiviral, contraceptive, and antifungal agents. Kirschner described a pH neutral bioadhesive drug delivery system suitable for modified delivery of a therapeutically active material in the vaginal cavity. The vaginal drug delivery system comprises an essentially pH neutral emulsion having globules with two phases, an internal water-soluble phase and an external water-insoluble phase or film. The water-soluble interior phase contains a therapeutically active drug or drugs. The particle sizes of the globules are ranging about 0.1 - 100 microns. The therapeutically active drug is selected from the group consisting of antifungal agents, antibacterial agents, antimicrobial agents, antiviral agents, spermicides, hormone agents, antitrichomonial agents, antiprotozoan agents, antimycoplasm agents, antiretroviral agents, nucleoside analogues, reverse transcriptase inhibitors, protease inhibitors, contraceptive agents, sulfadrugs, sulfonamides, sulfones, hygiene agents, probiotic agents, vaccine agents, antibody agents, peptide agents, protein agents, polysaccharide agents, nucleic acids, plasmids, liposomes, carbohydrate polymers, transgenic bacteria, yeast, chemotherapeutic agents, steroid agents, growth enhancing agents, libido enhancers, androgenic substances, chitin derivatives, environment modifying agents such as pH modifiers, and mixtures and combinations of them. The excipient is selected from the group consisting of lubricants, cleansing agents, deodorizers, humectants, emollients, plasticizers, binders, emulsifying agents, stabilizing agents, solvents, bioabsorbable materials, antioxidants, solubilising agents, antimicrobial preservatives, diluents, glidants, suspending agents, extended-release agents, coating agents, adsorbents, disintegrants, chelating agents, and mixtures and combinations of them.

5) Vaginal pessaries:

Pessaries can be easily applied to the vagina. Hydrated bioadhesive vaginal suppository formulations are formed of one or more hydrophilic polymers, such as sodium carboxymethyl cellulose, polyacrylic acids or polyacrylates,a pessary or suppository base, water (30 % by weight of the formulation) and an active ingredient. Brummer *et al* described an invention relates to a novel method of manufacturing a lyophilized suppository composition wherein suppository is made *in situ* in an applicator cartridge. In this patent cellulose ether compound was selected from the group consisting of hydroxypropylmethylcellulose, sodium carboxymethylcellulose, methylcellulose, Hydroxyethylcellulose, hydroxypropylcellulose, Hydroxyethylmethylcellulose, hydroxyethylethylcellulose, hydroxypropylethylcellulose, Carbopol, polyvinyl alcohol and derivatives thereof, dextran, chitosan and its derivatives, starch and its derivatives, polyacrylamides, polyacrylates, agar, collagen, fibronectin, alginic acid, pectin, hyaluronic acid or mixtures of them. Miconazole nitrate was used as the active ingredient. Bromocriptine is formulated as vaginal suppositories. In order to achieve bioadhesivity the formulation contains carbomer (Carbopol 974P) or chitosan. Manufacturing of this new formulation in a suppository form to be suited for vaginal as well as rectal application. Misoprostol is commonly used in obstetric practice for controlling post-partum and post-abortive bleeding and leading in labor or abortion whenever indicated. Vaginal suppositories containing misoprostol have been developed by Darwish [106]. The suppositories contain one poloxamer to enhance absorption and at least one bioadhesive compound. A mucoadhesive formulation for the treatment of vaginal infections has been assigned to Embil *et al*. The invention provides pharmaceutical compositions in the form of pessary for topical administration in treating vaginitis. This composition contains at least one triazol compound active against *Candida albicans*, and at least one additional anti-vaginitis medicament, preferably an anti-fungal, antiprotozoal and/or anti-bacterial agent. The formulation includes a mucoadhesive agent to promote adhesion to the vaginal mucosa. The use of bioadhesive may also provide for controlled release of the active agents. The bioadhesive agent

is selected from poly (carboxylic acid- containing) based polymers, cellulose derivatives (hydroxypropyl cellulose,

hydroxypropyl methyl cellulose), gums, clays, polysaccharides, carbohydrates and polypeptides, glycosaminoglycans, salts of alginic acid, scleroglucan, polyvinyl polymers, polysiloxanes, polyethers, polyalkylene oxides and glycols, polyalkoxys and polyacrylamides, xanthan gum,

Chitosans, and derivatives and salts of these. The bioadhesive agent comprises an *in situ*-gelling mucoadhesive, e.g. an in situ-gelling mucoadhesive polymer (glyceryl monooleate), or a thermoreversible gel (enzymatically treated starch). The composition is formulated for controlled, e.g. rapid and/or delayed (sustained) release of the active medicaments. The controlled release is provided by solid lipid nanoparticles. Pather *et al.* described an effervescent formulation. The pharmaceutical compositions of this invention comprise rectally and vaginally administerable dosage forms that contain effervescent agents as penetration enhancers for drugs. Effervescence occurs in the rectum or vagina, once the dosage form is administered or at a predetermined time following administration. The effervescent agents can be used alone or in combination with pH adjusting substances, which further promote dissolution and absorption of the active ingredient. Bioadhesive polymers increase contact time between active ingredient and a mucosa layer of target area.

a) Modified vaginal pessaries:

The Syncro–Mate progestin-releasing vaginal pessary is fabricated by dispersing a progestin, such as fluorogestone acetate, in a pessary made of porous polyurethane sponge. The pessary can be readily inserted intravaginally and removed according to a predetermined schedule. The vaginal pessary has been redesigned with the aim of minimizing the loading dose, to overcome the matrix-type release and absorption profiles, and to improve systemic bioavailability. Work evaluating a cylindrical, drug-free polyurethane vaginal sponge coated by a laminate of fluorogestonecontaining silicone matrix and a drug-free silicone coating membrane was conducted. The system itself was modified and the release rates studied in vitro and in vivo. It was found that the drug-containing silicone layer needed to be in contact with thevaginal wall. A silicone coating did not result in adequate release rate. Also, as the surface area of the drug-containing silicone increased, the drug delivery rate increased. This effort has resulted in two new pessary designs (Fig. 3). Both types make use of the polyurethane sponge in the vaginal pessary as the mechanical support for vaginal insertion and retention, but thedrug reservoir is relocatedfrom the porous sponge matrix to a sheet-type rate-controlled silicone device that covers the circumferential surface of the sponge. The type I rate-controlled silicone device consists of a homogeneous dispersion of drug in a silicone polymer matrix. The type II drug-dispersing polymer matrix is sandwiched between two sheets of silicone polymer membrane to form a three layered laminate.

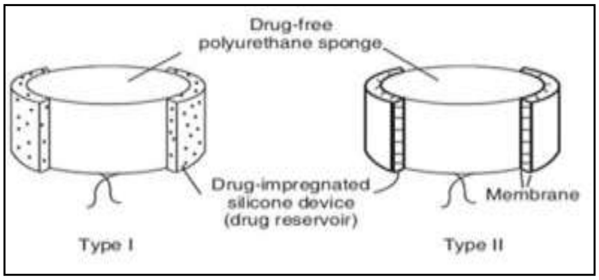

Figure 3: Newer progestin-releasing vaginal pessaries (Type I and II)

6) Creams, Foams and Jellies:

Many OTC vaginal products are available in these types of preparations. Common products include contraceptive creams, foams, gels, suppositories, sponges, and films that contain an active spermicidal agent, like non-oxynol-9 or octoxynol. Vaginal preps are a recent FDA-approved OTC treatment for yeast infections. For treatment of menopausal symptoms, estrogen products are available as vaginal creams. A relatively new product for postmenopausal women, Replens_, is available as a vaginal bioadhesive moisturizer. It has been shown to be as safe and effective as estrogen vaginal creams in increasing vaginal moisture, fluid volume, elasticity, and returning the pH to the premenopausal state. Low pH lactate gels have been dispensed for the treatment of bacterial vaginosis. Application of these gels leads to a disappearance of abnormal discharge and malodor, restores normal acidity, and facilitates recolonization with lactobacilli. Local treatment for vaginosis by the intravaginal delivery of antimicrobials may be preferred over an oral regimen, particularly during pregnancy. When the efficacy of a vaginal ring, which delivers a continuous low dose of estradiol, was compared with a vaginal cream, both formulations were found to be equally effective and safe in the treatment of postmenopausal women with urogenital atrophy symptoms.

7) Vaginal rings:

Vaginal rings provide a means of delivering a pharmacologically active agent to the systemic circulation at a controlled rate of release. The vaginal rings developed to date are primarily used for contraception and have been reviewed in the literature. Compounds delivered include medroxyprogesterone acetate (MPA), estradiol, norgestrel, levonorgestrel, combinations of progestins and estradiol, and combinations of progesterone and estrogens. Another area of interest is in the controlled delivery of prostaglandin for cervical ripening and induction of labour or pregnancy termination.After oral ingestion, MPA, reaches a peak plasma drug concentration rapidly within 2 h and declines over the next 22 h. On the other hand, intravaginal controlled delivery of MPA from a vaginal ring attains a steady plasma plateau within 4 h, which is maintained throughout the course of treatment until removal of the ring. The continuous ‘‘infusion’’ of drugs through the vaginal mucosa can prevent the possibility of hepatic GI first-pass metabolism and inefficient therapeutic activity resulting from the alternatively surging and ebbing plasma drug levels that occur with the intermittent use of oral dosage forms.Vaginal rings have been shown to be safe and effective for the delivery of estradiol and have been found to be more comfortable than a pessary. Local estradiol delivery via vaginal ring, vaginal cream, or suppositories, was reported to alleviate urogenital estrogen deficiency symptoms in postmenopausal women. In both cases, patients showed a strong preference for the vaginal ring over other vaginal dosage forms. Danazol, that was administered intravaginally also via the vaginal ring was found to be very effective in the treatment of pelvic endometriosis. Danazol was absorbed through the vaginal mucosa and reached the deeply infiltrating endometriosis via diffusion.

Vaginal rings are made of biocompatible silicone elastomers that consist of a drug-free core ring and a drug-containing coat. Vaginal rings are inserted and positioned around the cervix. Those designed for contraception, are kept intravaginally for 21 days and removed for 7 days to allow menstrual flow. The vaginal ring was redesigned due to frequent bleeding irregularities. The new generation of sandwich-type vaginal rings contains a drug-dispersed silicone polymer matrix is coated by a non-medicated silicone polymeric membrane. The design reduces the initial spike of drug release frequently observed in the first treatment cycle of vaginal rings for contraception. The concept of intravaginal dual administration of progestin and estrogen in combination was recently extended to the development of a combined contraceptive vaginal ring. This new design (Fig.4) is constructed from two drug reservoir compartments; the major compartment consists of a 3-keto-desogestrel loaded core, and the other, minor compartment consists of a core loaded with a combination of 3-keto-desogestrel and ethinylestradiol, a synthetic estrogen. These drug reservoir compartments are separated by two steroid-impermeable glass closures, as the partitions, and release the steroids at a fixed ratio through a rate limiting silicone membrane.

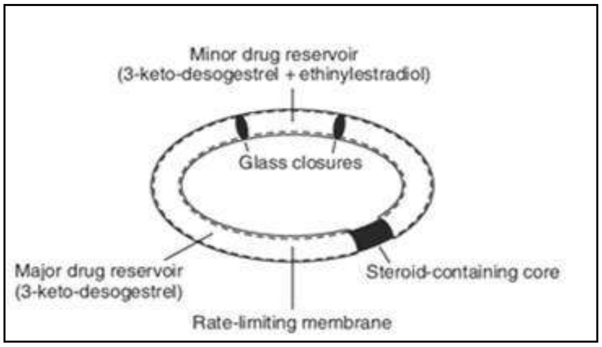

Figure 4: Contraceptive Vaginal ring

EVALUATION OF VAGINAL DRUG DELIVERY SYSTEMS:

A vaginal formulation must be evaluated by both invitro and invivo experimentation for various functional requirements.

a) *INVITRO STUDIES:*

Invitro studies include the determination of various physical and chemical properties of formulations, its bioadhesive and release characteristics. The bioadhesive strength has been estimated with the help of assemblies based on the principle of measuring tensile strength or shear stress required to separate the formulation from the vaginal mucosa. Disintegration or dissolution tests, uniformity of content or weight are some of the official tests for pessaries. Membrane diffusion studies, microbiological methods and a vaginal dissolution tester can determine release characteristics of a drug from a formulation. To stimulate the vaginal environment, different compositions of vaginal fluid have been used for invitro testing of vaginal formulations. A chemically defined that stimulates female genital tract secretions that can support the growth of vaginal microflora has also been reported.

b) *INVIVO STUDIES:*

Invivo studies are carried out of the assessment of efficacy, distribution, spreading and retention of formulations in the vagina. In the process of development of a vaginal formulation, various animal models such as sheep, rat, rabbit, rhesus monkey, macaque monkey, dog and mice have been used. The rabbit is the recommended model for vaginal irritation studies. There is significant species variability in the anatomy and physiology of the vagina of different animals. Because of interspecies differences, the evaluation of vaginal formulations in human subjects is desirable. The rate and extent of drug release can be determined by

i. Monitoring quantities of systemically absorbed materials
ii. Measuring the pharmacological activity, and
iii. Analysis of vaginal lavage

Gamma scintiography is a valuable method for assessing the distribution, spreading and retention of vaginal formulations in sheep and human females. Coloscopy has also been used for direct invivo visualization and analysis.

APPLICATIONS OF VAGINAL DRUG DELIVERY SYSTEMS:

1) This route of drug administration is useful for vaginal immunization.

2) Multi-cycle administration of vaginal contraceptive rings.

3) Effective route for the treatment of HIV infection.

4) Effective route for the treatment of local fungal infection.

5) Effective for the delivery of hormones.

DRUG CANDIDATES FOR VAGINAL ADMINISTRATION:

1. Antimicrobials
2. Anticancer agents
3. Prostaglandins
4. Spermicides
5. Steroids
6. Proteins and peptides
7. Vaccines, antigens and gene delivery
8. Anti-inflammatory agents

Active medicament	**Dosage form**
Clindamycin phosphate	Cleocin (Cream)
Progesterone	Crinone (Gel)
3-ketodesogestrol ethinylestradiol	CCVR (Vaginal ring)
Estrogen	Estring (Vaginal ring)
Naphthalene-2-sulphonate	Pro 2000b (Gel)
Butaconazole	Femstat One (Emulsion)
Nanoxynol-9	LASR (Suppository)

Table 2:- Marketed Preparations Of Vaginal Drug Delivery Systems:

CURRENT & FUTURE DEVELOPMENTS:

Vagina is an important site for the delivery of drugs, particularly contraceptives for ages. As the advacement in pharmaceutical technology, the new delivery systems are taking the place of the traditional delivery sytems such as suppositories, tablets, creams and gels. The consideration of women's opinions on vaginal products is also important for the development of acceptable dosage forms and better compliance.

Mucoadhesive systems have a unique place among the different new delivery systems. Mucoadhesive polymers have been successfully applied for systemic and local vaginal drug delivery. Acrylic acid polymers (Carbomer or polycarbophil) and cellulose derivatives, such as hydroxyethylcellulose, Hydroxypropylcellulose and hydroxypropyl methylcellulose have been widely used as mucoadhesive polymers for the preparation of mucoadhesive vaginal drug delivery systems. There are a number of patents based on both group of polymers. In particular it can be said that polyacryclic acid polymers are excellent multipurpose vehicles for vaginal drug delivery. The current systems are used for vaginal lubrication, contraception, vaginal infections, labour inducement and infertility. Among the possible topical compositions, gels present notable advantages in comparison with other types of pharmaceutical products, such as good compliance in administration by the patient and ease of distribution of the pharmaceutical product on the surface of the vaginal mucosa. Gels in particular, on account of the high water content in their structure, present the further advantage of a hydrating and lubricating action, which is particularly useful in pathological situations characterised by dryness of the vaginal mucosa.

Despite the numerous vaginal gel formulations patented, relatively few gel preparation are commercially available. Most of them are used for contraception and moisturazition of vagina. Up to our knowledge, there is no commercially available mucoadhesive vaginal tablet formulations present.

When present patents checked for mucoadhesive vaginal drug delivery systems it can be noticed that there are many patents available having these key words. Moreover, as it is a general complain, these patents claim several different things. For instance, some of them have many active and inactive ingredients with broad range of quantity or one patent have a claim of even different dosage forms in the same application such as gel, ointment, tablet, film or suppository etc. for vaginal application. This claims obviously written just to increase the coverage of the patents to make breaking attempts of patent impossible. It is really difficult to find precise patents and precise claims. All claims were found to be exaggerated much. It is therefore difficult to focus on precise aim. It may be just normal for applicant to increase the border of their patent coverage but, it also makes new applications difficult and many times impossible. Although there are limited numbers of commercially available mucoadhesive vaginal drug delivery systems, in the near future mucoadhesive vaginal drug delivery systems will gain more significance due to the increase in sexually transmitted diseases such as HIV.

Brand name	**Gelling agent**	**Active substance**	**Usage**
Acid jelly®	Tragacanth, acacia gum	Oxyquinoline sulphate, Ricinoleic acid, Acetic acid	Maintainence of the vaginal acidity
Gynol 11®	Sodium carboxy methyl cellulose	Nonoxynol-9	Contraceptive
Prostin E2®	Colloidal silicon dioxide	Dinoprostone	Labour inducer
Replens®	Polycarbophil and Carbopol®974P	-	Vaginal mosturizer
Metrogel vaginal®	Carbopol®974P	Metronidazole	Bacterial vaginosis

Table 3:- Marketed mucoadhesive vaginal gels

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PARENTERAL CONTROLLED DRUG DELIVERY SYSTEM

Maravaniya Pathikkumar, Shaikh Aamir

INTRODUCTION:

Number of drug delivery systems has been developed over the years, parenteral drug delivery system being one of them. Parenteral drug delivery refers to administration by injection which takes the drug directly into the tissue fluid or blood without having to cross the intestinal mucosa.

The limitations of oral route are circumvented. Action is faster and surer (valuable in emergency). Gastric irritation and vomiting is not provoked. It can be employed even in unconscious, uncooperative or vomitose patient. There are no chances of interference by food or digestive juices. Liver is also bypassed by this route (Tripathi 2006).

But this route specifically requires that the drug delivery system should be sterile, besides being invasive and painful, assistance of other person often being required (though self injection is possible, e.g. insulin by diabetics), there are chances of local injury and being more risky. Once administered, the action is difficult to revert back in case of side effects or toxicity. The different parenteral routes are subcutaneous, Intravenous, Intramuscular, Intra dermal and Intraperitonial.

Parenteral Controlled Drug Delivery System:

The Parenteral administration route is the most effective and common form of delivery for active drug substances with poor bio-availability and the drugs with a narrow therapeutic index.

For this reason, whatever drug delivery technology that can reduce the total number of injection throughout the drug therapy period will be truly advantageous not only in terms of compliance, but also for potential to improve the quality of the therapy.

Advanced drug delivery technology that can reduce the total number of injection throughout the drug therapy period will be truly advantageous not only in terms of compliance, but also fo potential to improve the quality of the therapy. Such reduction in frequency of drug dosing is achieved, in practice, by the use of specific formulation technologies that guarantee that the release of the active drug substance happens in a slow and predictable manner (Raghanaveen 2009).

For several drugs, depending on the dose, it may be possible to reduce the injection frequency from daily to once or twice monthly or even less frequently. In addition to improving patient comfort, less frequent injection of drugs in the form of depot formulation smoothes out the plasma concentration time profiles by eliminating the peaks and valleys. Such smoothing out of the plasma profiles has the potential to not only boost the therapeutic benefit but also to reduce unwanted events and side effects (Alessandro 2004) (Heller 1990). The development of new injectable drug delivery system has received considerable attention over the past few years[2]. This interest has been sparked by the advantages this delivery system possess, which include ease of application, localized delivery for a site specific action,prolonged delivery periods, decreased body drug dosage with concurrent reduction in possible undesirable side effect common to most forms of systemic delivery and improved patient compliance and comfort.The release can either be continuous or pulsatile depending on the structure of the device and the polymer characteristics, continuous release profiles are suitable to generate on ‘infusion like’ plasma level time profile in the systemic circulation without the necessity of hospitalization.

Economically, only well-trained personnel can administer injections, and if frequency of administration is reduced, the cost of therapy is decreased and time is saved. In addition to improving patient comfort, less frequent injection of drugs in the form of depot formulation smoothes out the plasma concentration time profiles by eliminating the peaks and valleys. Such smoothing out of the plasma profiles has the potential to not only boost the therapeutic benefit but also to reduce unwanted events and side effects (Raghanaveen 2009).

In principle, there are three ways to achieve prolonged release of parenteral dosage form. These are- pharmacological, chemical, and physical methods. (pharmatips)

Pharmacological methods

This includes intramuscular or subcutaneous administration instead of intravenous; the simultaneous administration of vasoconstrictors (adrenalin in local anaesthetics; ephedrine in heparin solutions) and blocking the elimination of drugs through the kidneys by simultaneous administration of a blocking agent, such as probenecid with penicillin or p-amino salicylic acid.

Chemical methods

These include the use of salts, esters, and complexes of the active ingredient with low solubility.

Physical methods

These include the selection of the proper vehicle, thereby giving prolonged release (use of oleaginous solutions instead of aqueous solutions); the addition of macromolecules that increase viscosity (CMC, NaCMC, PVP, tragacanth, etc.); the use of swelling materials to increase viscosity in oleaginous solutions (aluminium monostearate); the additions of adsorbents; the use of solutions from which, upon administration, the drug is precipitated when it contacts body fluids; the use of aqueous and oleaginous suspensions; and the use of implants (Robinson 2003).

Pareneteral Controlled Drug Delivery System:

A. AQUEOUS SOLUTION:

1. HIGH VISCOSITY PRODUCTS:

Increasing viscosity of the vehicle, the diffusion coefficient of the drug will be reduced, thereby delayed in drug transport. Viscosity agents:

1. Methycellulose
2. Sodium carboxymethyl cellulose and
3. PolyvinylPyrollidine.

EFFECTS OF VISCOSITY:

Increase the viscosity of the medium not only decrease the molecular diffusion but also localize the injected volume thus the absorptive area is reduced and the rate drug transfer is better controlled. Incorporating gelling agents like alluminium monostearate into oil solutions. The actual viscosity is close to that of water, thus offering little diffusion resistance.

2. COMPLEX FORMATION:

The role of plasma protein and tissue binding in prolonging drug action is well known. Forming a dissociable complex of a drug with such macromolecule as methycellulose, sodium carboxyl Methycellulose and polyvinyl pyrollidone for intramuscularadministration.

Constant fraction of drug is complexed and that only free drug is absorbed, the absorption rate dcldt may be expressed as

$$d[c]/dt = -k f [c]$$

where, k = is the absorption rate constant

f = fraction of drug that is the free

[c] = Is the total concentration of drug at the absorption Site.

$$F = l/(1+ Ka [m]) \quad (2)$$

In which Ka is very much large than 1.

$$F = l/Ka [M] \quad (3)$$

Various degree of control can be exercised by selecting the appropriate type and concentration of macromolecule since such each drug macromolecule pair has a characteristic association constant and since the free drug concentration is inversely proportional to macromolecule concentration. Complex between drug molecule and macromolecules, complexes can also be formed between drug molecules and other small molecule such as caffeine. Contrast to complexes with macromolecules, complexes with small molecules can be absorbed. This phenomenon from the standpoint of alteration of physiochemical properties of the dug molecule upon complexation, the effect of the complexing agent on the barrier, and the stability constant of molecular complex.

DISADVANTAGES:

The small association constant that usually exist between small molecules. Complex formed between large drug molecules, such as peptide hormones and small complexing agent such as tannic acid fall into the same category.

1. Protamine zinc insulin
2. ACTH zinc tannate
3. Cynocobalamin zinc tannate.

B. OIL SOLUTION:

It less elegent mechanism to achive parenteral controlled release is through the use of oil solutions. Drug release is controlled by partitioning of drug out of the oil into the surrounding aqueous medium. Dynamic equilibrium between drug in the oil phase and that in the aqueous phase with characteristic constant, the apparent partition co-efficient K.

K = drug concentration in oil /drug concentration in water

$$K = [Do] \quad [Dw] \qquad (I)$$

Where the drug concentration in water refers. To both ionized and unionized species of the drug. The volume of the oil phase Vo and that of aqueous phase Vw that total amount of drug Dt in the system at any time can be represented by

$$Dt = [Dw] \quad [K \quad Vo \quad +Vw] \qquad (2)$$

The fractional amount of the drug

$$F \quad =1 \quad (K\alpha+1)$$

$$\text{Where,} \quad a = Vo/Vw \qquad (3)$$

Since absorption is driven by concentration, not amount and expression for the fractional concentration of drug that is in the aqueous phase f

$$f = (1 \quad +a)/(l \quad + \quad K\alpha) \qquad (4)$$

There limiting cases,

Case- I For $\alpha < < 1$, one obtains, $f = 1 / (1 + K\alpha)$

Case -2 For $\alpha > > 1$ one obtains, $f = \alpha / (f + K\alpha)$

Case -3 provided K is sufficient large obtains from case -2 $f' = l/K$

1. The fraction of drug that is available for absorption is controlled by the partition co-efficient and the ratio of the volume of two phase' a.
2. That is remain constant as long as a is constant.

Vw is constant since it is physiological parameter. So that the value of α is controlled solely by the volume of solution injected. Drug absorption occurs via the aqueous phase an expression describing the absorption rate {d [c] / dt} similar to that for the complex formation.

$$D [c] /dt = - Ka \ f' \ [c]$$

Ka f ' can be obtain from this relationship. Usually an estimate of Ka is available so, that f can be determined.

Given value of α, K can be estimated from the rearranged from eq.

$$K = [(I \ -f')/Vo] \quad (f'/Vw) \quad +1/f$$

LIMITATIONS:

Limitation of this method in evaluating K are immediately obvious.The volume of interstitial fluid at the injection site is till defined Oil can be absorbed so, that Vo is continuosly changing with time. IMPORTANCE OF " K " :K which is function of the drug involved and the oil selected.

C.SUSPENSION:

DEFINITION:

Parenteral suspension are dispersed, heterogeneous systems containing insoluble drug particle which, when resuspended in either aqueous or vegetable oil vehicles.

They should be sterile, pyrogen free, stable, resuspendable, syringable, injectable, isotonic & non-irritating.

Because of above requirements

injectable suspensions are one of the most difficult dosage forms to develop in term of their stability, manufacture & usage.

The parenteral suspension may be formulated as already to use injection or require a reconstitution step prior to use. They are usually administered by either subcutaneous (S.C.) or intramuscular (I.M.). Never suspension delivery systems containing drug in microparticulate or nanoparticle can be injected by intravenously or intraarterially.

These suspensions usually contain between 0.5% and 5.0% solid & should have particle size less than 5 micrometer for I.M. or S.C. administration. Certain antibiotic preparation (For example procaine Penicillin G) may contain up to 30 % solids.

Parenteral suspensions are developed due to following points:

The drugs, which are insoluble to be formulated as a solution.
For the drug which are more stable as suspended solid than in solution.
There is need to retard or control the release of drug from suspension.
The main advantages of preparation of parenteral suspension are……
It is better for the therapeutic use of drugs that are insoluble in eonventionalsolvents.
In this dosage form there is increased resistance to hydrolysis & oxidation as drug is present in the solid form.
Formulation of controlled release drug id this dosage fonn.
There is elimination of hepatic first pass effect.

DISADVANTAGES:

Difficulty in formulation: (R.L. 2003) (Ravivarapu 2000)
Parenteral suspension also limit the formulator in what ingredients are parenterally acceptable as suspending agent, viscosity induce, wetting agent, stabilizers and preservative.

Difficulty in manufacturing special facilities a required to maintain aseptic condition for manufacturing processes such as Crystallization
- Particle size reduction
- Wetting
- Sterilization

The stabilization of suspensions for the period between manufacture &use present a number of problems .e. g. solid gradually settle &may cake, causing difficulty in redisdersion prior to use.) Due to above reason it is discomfort to use.
- Maintenance of physical stability is very difficult in this dosage form.
- There may be chances of non-uniformity of dose at time of administration.

Some of the official preparations are;

a. Sterile ampicilling suspension USN ' 95 dispense as powder which is to be reconstituted at time of administration.
b. Sterile Aurothioglucose suspension USP 95 - vegetable oil suspension.
c. Tetanus toxoid adsorbed USP '95 - Aq. Suspension.
d. Betan ethasone apetate suspension USP 95 -Aq. Suspension.
e. Insulin Zinc suspension USP 95 Aq. suspension.
f. Procaine penicilling suspension IP' 96.

D. EMULSIONS:

Besides use in topical drug delivery, emulsions been used as drug vehicle both orally and parenterally While not matching topical emulsions more prodrugs has been made with parenteral than with oral emulsion.

1. OIL-IN-WATER AND WATER-IN-OIL EMULSIONS:

The elevation and prolongation of antitoxin levels obtained by oily vehicles before injection using emulsified influenza.

MECHANISM OF ACTION:

One mechanism by which the adjuvant action is brought about is the slow continuous release of antigen from the emulsion.
Rate of release is treated by

Rate =4p (a022 D⌂ct/d)1/2 DAc

Where, a0 is the initial radius of the droplet
D is the diffusion co-efficient
d is the density of the solute
⌂c is the difference in concentration between the surface of the droplet and bulk phase.

The growing interest in using water-in-oil and oil-in-water emulsions as vehicles for parenteral drug delivery id the development of multiple emulsions for controlled drug release.

CONTROLLED PARAMETERS:

a. Primary water-in-oil emulsion

- Internal phase volume
- Concentration of the emulsifier
- Osmolarity of dispersed phase

b. Multiple emulsions

- Formulation
- Stability
- Drug release

2. MAGNETIC EMULSIONS:

The emulsion described is a magnetically responsive oil-in-water type emulsion with capacity to localize the chemotherapeutic agent.

The magnetic emulsions consist of ethyl-oleate-based magnetic fluid as dispersed phase and casein solution as continuous phase.

The magnetic emulsions had high retention by magnetic field in vitro and aver intravenous injection in the rate.
The magnetic emulsions was localized at the lungs by application of an electromagnet one the chest.

E. LIPOSOMES:

LIPOSOMES IN PARENTERAL DRUG DELIVERY:

- To avoid quick metabolism.
- E.g. Chloroquine, superoxide, dismutase etc.
- To protect patient from side effect.
- E.g. Doxorubicin on heart muscle.
- To reduce hemolytic effect and irritation by intradermal Subcutaneous and lM injection.
- The most advanced application of liposome based therapy is in the treatment of systemic fungal infections, especially with amphotericin B.
- l,iposomes are also under investigation for treatment of neoplastic disorders.
- E.g.: encapsulation of known antineoplastic agents such as doxorubicin and methotrexate,

- Delivery of immune modulators such as N-acetylmuramyl-L-alanine-D-isoglutamine,
- Encapsulation of new chemical entities that are synthesized with lipophilic segments tailored for insertion into lipid bilayers.

Modulation of Drug release rate

Boman et. Al. Showed that the therapeutic activity of vincristine encapsulated in GM 1- containing liposomes could be significantly boosted by improving the drug retention property of liposome leading to slow release GM I: Monoasioganglioside

LIPOSOME STRUCTURE:

- Bi layered structure of phospholipids and cholesterol
- Capable of entrapping both water sol. And lipid soluble drugs
- Can alter biodistribution, protect drug and body from each other
- Special liposomes usable for target delivery

DEMERITS OF INJECTABLE LIPOSOME:

- RES- Mediated Clearance of liposomes
- Critical in determining liposome half life
- Site of liposome clearance-Liver & spleen
- Physiologic role of Macrophages
- Hepato-Splenic uptake of liposomes.
- Inverse correlation of blood & RES levels of Liposomes

APPLICATION OF LIPOSOMES:

Liposomes in the treatment of Leukemia

1. In the case of iv injected leukemia model, in which no extravasation step may be needed for liposome targeting to tumor cells
2. In the study using GM-I LS containing the drug Ara-C and the rapidly growing L1210 leukemia model, Allen et. AI reported that the faster drug leakage rate was directly correlated with efficacy.

F. NIOSOMES:

1. Differs from liposomes in having surfactant in place of phospholipid
2. Size in smaller than MLVs
3. Easy to prepare and stable.
4. Niosomes also interact with cell surface
5. Acceptability mainly depends on the surfactant selection (For toxicity)

APPLICATION OF SLN PARENTERAL:

1. Ranges from intra articular to intra-venous.
2. Both stelth and non-stelth SLN are used I.V.
3. Prolonged plasma levels achieved with paclitaxal in-vivo
4. Increased uptake observed in brain, but low uptake in liver and spleen.
5. Potential application to treat arthritis- corticoid SLN injected to joints would phagocytosis and release drug inside reducing hyperactivity.

APPLICATIONS OF SLN NEW ADJUVANT FOR VACCINE:

1. To enhance the immune response and safety in
2. Comparison the traditional adjuvant like aluminum hydroxide
3. Long lasting exposure to immune system due to slow degradation (Still reducible using stealth SLN)
4. Potentiality is being worked out as effective vaccine delivery agent.

G. MICRO-EMULSIONS:

1. Transparent, thermo-dynamically stable emulsion suitable as injectable even as IV.
2. Accommodates fat soluble drugs: Cyclosporine
3. Avoids irritation and pain: E.g.: Propofol infusion
4. Reduces toxicity: Paclitaxol

Micro-emulsion of cyclosporine as IV infusion

1. Cyclosporin A is widely used as an immunosuppressant e.g., in the prevention and treatment of gram rejection following organ transplant and of graft versus host disease, e.g., following bone marrow transplant.
2. At higher dosages, however, it may affect kidney and liver function.
3. Moreover, cyclosporin A is difficult to formulate, as it is essentially insoluble in most pharmaceutically acceptable solvents.
4. Aqueous pharmaceutical systems, and its oral bioavailability in most formulations are variable.

H. MICROSPHERES:

1. Microcapsules
2. Micromatrices

1. MICROCAPSULES:

They are spherical particles containing drug concentration in the center core, which is eveloped by polymeric wall(rate controlled membrane)

2.MICROMATRICES:

Micromatrices are solid, spherical solid particles containing dispersed drug molecules either in solution or in crystalline form. They are part of homogenous monolithic drug release system. The monolithic microcapsules are some time called microspheres.

They are made up of polymeric, waxy or other protective materials, that is biodegradable synthetic polymer and modified natural products such as starches, gums, protein, fats and waxes. The natural polymers include albumin and gelatin . Synthetic polymers include polylactic acid, polyglycolicacid and EC, polysteres, polycaprolactone, and polyacrylarrtide .MIS are small in size and therefore have large surface to volume ratios.

At the lower and of their size range they have colloidal properties. The interfacial properties of MIS are extremely important, often dictating their activity.

ADVANTAGES OF MICROSPHERES:

- Taste and odor masking
- Conversion of liquids to solids
- Protection of drugs against environment
- Improves the flow property of powders
- Have controlled release
- Have targeting property
- As microspheres are multiple unit product, ready distribution over a large surface area
- Delocalisation of the total dose in the GI tract
- Reduce side effects
- The drug release rate will be less dependent on gastric transit time
- The drug -loaded microspheres are more resistant to fracture.

DISADVANTAGES OF MICROISPHERES:

- Burst-effect
- Inadequate shelf life of sensitive pharmaceuticals.
- Non-reproducible
- Costly
- Difficult to scale up

MAGNETIC MICROSPHERES:

MICROSPHERES FOR TARGETING DRUGS:

It is tissue or cellular localization that increases the therapeutic index by at least half an order of magnitude. Targeting causes drug level in liver, spleen, bone marrow, kidney and other major sites of toxicity.

Advantages:

- Cross microvascular barriers independent to Endothelial status.
- Protect the drug, blood cells, and Endothelium during transit.
- Deliver up to 60% dose to target tissue.
- Drug available in controlled fashion.
- Reduce concentration of free drug.
- Minimize damage to normal tissue cells.
- Decrease the dose

Disadvantages:

- Expensive
- It require specialized microspheres and magnets
- Treatment of multiple body regions require sequential targeting
- In long term deposition of magnetite (Fe304)

Drug candidates:

- Dangerous drug and labile which causes toxicity when circulated in blood stream.
- Expensive drug, and hardly 0.1% have action and 99.9% wasted in body
- If drug have life threatening toxicity then MIS is alternative formulation.

I.IMPLANTS:

INTRODUCTION:

Lafarge first introduced the concept of implantable therapeutic system for long term, continuous drug administration in 1861 with the development of a subcutaneous implantable drug pellet. The technique was used to administered crystalline hormone in form of solids steroids pellets. Implantable system is capable of continuous secretion ion hormones from active gland.

Implant represents novel approach in the use of solid dosage forms as parentral product. Implants are insert under the skin by cutting and stitching it alter insertion of' the sterile tablet which is cylindrical, rod and ovoid shaped and more than 8 mm in length. The sterile tablets consisting of the highly purified drug, compressed without excipients.if, intended for subcutaneous implantation in the body. Implantations tablet have been lately replaced by other dosage form such as diffusion control silicon tube, tilled with drug or biodegradable polymer.

Magnetically controlled capsules are imaginative form of' implant; such capsules are implanted in upper thigh at a depth 0.5 cm. This type of capsules is 1.2 cm long and 0.6 cm in diameter and made by moulding polycarhonatcs and also suitable for presentation of contraceptive hormone.

The subcutaneous release rate of steroid form of pellets implantation was found to be slowed down and hormonal activities was prolonged by dispersing the steroid in cholesterol matrix during pellets fabrication. The clinical use of implantable pellet for human health care has declined in recent year. The fact has triggered the research and development of novel, controllable and impalntable therapeutics system to replace pellet for long term, continous subcutaneous administration of drug.

In this case few of the traditional implant is use including desoxycorticosterone acetate, estradiol and testosterone. Some of the recently approved implantation product includes biodegradable and non-biodegradable polymer. Several impalntable forms for a prolonged drug delivery are in commercial use.

ADVANTAGES:

- Improved control of drug level at the specific site of action.
- Preservation of the medication that are rapidly destroyed but the body.
- Immediate removal of implants is possible in contrast to conventional drug delivery system in case of extreme allergies or side effect due to the dnig already administered.
- Less fluctuation in plasma drug level during therapy.
- Possible reduction in therapy costs because patient care and the potentially lower drug dose required.
- Improved special compliance.
- Administration of drugs with short biological half-life may be facilitated.
- Minimal harmful side effect of systemic administration through local therapy.

DISADVANTAGES:

- Toxicity or lack of biocompatibility of the material used for the implant.
- Harmful byproduct may be formed from the system, particularly for biodegradable types.
- Dose dumping and variable imprecise drug release may occur if not formulated properly.
- Pain and discomfort may be caused by the presence of implant.
- These system can be more expensive than the conventional dosage form.
- Most impalntable controlled drug delivery system requires minor surgery to implant and to remove from the administered site, if it is not biodegradable type.
- Possibility of the tissue and the body reaction to implant.
- Danger of toxic effect in case of leakage or burst release of drug.

MECHANISM OF DRUG RELEASED FROM IMPLANTABLE THERAPEUTIC SYSTEM:

A. CONTROLLED DRUG RELEASED BY DIFFUTION;

- Membrane permeation - controlled drug delivery.
- Matrix diffusion -- controlled drug delivery.
- Micro - reservoir dissolution controlled drug delivery.

B. CONTROLLED DRUG RELEASE BY ACTIVATION:

- Osmotic pressure activated drug delivery.
- Magnetism - activated drug delivery.
- Ultrasound activated drug delivery.
- Vapour pressure activated drug delivery.
- Hydrolysis activated drug delivery.

J. INFUSION DEVICES:

These are also implantable devices but are versatile in the sense that they are intrinsically powered to release the medicament at a zero -order rate and the drug reservoir can be replenished from time to time. Depending upon the mechanism by which these implantable pumps are powered to release the contents, they are classified into following types;

1. Osmotic pressure activated drug delivery systems
2. Vapour pressure activated drug delivery systems
3. Battery powered drug delivery systems.

ADVANTAGES:

1. Osmotic pump can be used as a useful experimental tool to determine important pharmacokinetic parameters of new drugs, which ultimately find use in the development of an optimized delivery system,
2. Osmotic systems deliver the drug at zero order release kinetic, so they are superior to older sustained release technologies in many instances because of better control over their in-vivo perfomance is possible.
3. Drug release from osmotic system is independent of variation in environment pH and hydrodynamic conditions.
4. It is possible to attain substantially higher release rates than with diffusion base drug delivery systems.
5. Osmotic systems are able to deliver very large volumes.
6. In osmotic system reformulation is not required for different drugs.

DISADVANTAGES:

1. Osmotic system can be much more expensive than conventional systems.
2. Quality control of osmotic systems is more complicated than most conventional tablets.
3. Implantation is required for osmotic implants.
4. The drug which are unstable in solution, that may be inappropriate because the drug remains in solution form for extended periods before release.

OTHER PARENTERAL CONTROLLED DDS:

THE ATRIGEL DRUG DELIVERY SYSTEM

The Atrigel system is a proprietary delivery system that can be used for both parenteral and sitespecific drug delivery. Atrigel system was initially developed by Dunn and co-workers at Southern Research Institute in Birmingham, Alabama in 1987. The technology was licensed to Vipont Research Laboratories (which later became Atrix Laboratories) for the sub gingival delivery of antimicrobials to treat periodontal disease (Dunn 1990). This system serves many advantages over conventional methods of drug administration including tablets, capsules etc. These include-

Compatibility with a broad range of pharmaceutical compounds: Water soluble and insoluble compounds and high and low molecular weight compounds like peptides and proteins, vaccines and natural products can be easily administered by Atrigel systems.

Less invasive technique: The application is less invasive and painful compared to implants, which require local anaesthesia and a small surgical intervention.

Direct delivery to a target area: Thus helps in achieving higher drug concentrations at the desired site of action to minimize systemic side effects.

Protection of drug: Development of an Atrigel drug delivery system of a protein drug helps in preventing denaturation of protein in body fluids.

Sustained drug release: Helps in reduction of dose, achieve release for extended periods, so there is increase in patient compliance, important for those protein drugs having narrow therapeutic indices.

Biodegradable and biocompatible: Atrigel system is made of biodegradable polymers and biocompatible solvents so do not require removal.

Economic factors: Microspheres have to be washed and isolated after preparation; operating expenses for the production of in situ forming applications are marginal, thus lowering investment and manufacturing costs. The technology for the Atrigel system is protected by 33 patents in the United States and 35 patents in the rest of the world. These patents cover the basic technology as well as process improvements (D. B. Patel 2010).

FORMULATION AND DEVELOPMENT:

The formulation of these systems includes the dissolution of the water insoluble biodegradable polymer into a biocompatible solvent. The drug is next added to the solution where it dissolves or forms a suspension. This drug/ Polymer mixture is then easily and conveniently injected into the body where it forms a solid implant inside the tissue. Most commonly used polymers are poly (dl-lactide), lactide/glycolide copolymers, and lactide/caprolactone copolymers because of their degradation characteristics and their approval by the Food and Drug Administration (FDA).These offer advantage that breakdown products are natural, biocompatible so no problem of toxicity. Various rates of biodegradation can be obtained depending on type of polymer, there combination and ratio (Jain 2008). Polymer concentrations ranging from 10 to 80% by weight are used for preparation of Atrigel drug delivery system (Dunn 1990).

The low molecular weight polymers at low polymer concentrations can be easily injected into the body using standard needles, and they can also be aerosolized for spray applications. The high molecular- weight polymers at high polymer concentrations may be used as gels or putties that can be placed into sites in the body where they solidify and provide support. Some examples are depicted in table 1.

Polymer	**Time of biodegradation**
Poly Lactide	28-24 Months
Poly dl- Lactide	12-16 Months
50:50 Lactide/Glycolide	50-60 Days
85:15 Lactide/Glycolide	5 Months

Table 1. Biodegradation time of different biodegradable polymers (Jain 2008)

The solvents employed in the Atrigel system to dissolve the polymers range from the more hydrophilic solvents such as dimethyl sulfoxide, *N*-methyl-2-pyrrolidone (NMP), tetraglycol, and glycol furol to the more hydrophobic solvents such as propylene carbonate, triacetin, ethyl acetate, and benzyl benzoate. The most frequently used solvent is NMP because of its solvating ability and its safety/toxicology profile. A Drug Master File on this solvent has been filed with the FDA (Spiegel A.J. 1963)

When this formulation is injected into the body the water miscible organic solvent dissipates and water penetrates into the organic phase. This leads to phase separation and precipitation of the polymer forming a depot at the site of injection as shown in Fig 1 (R.L. 2003)- (Moyer 2000).

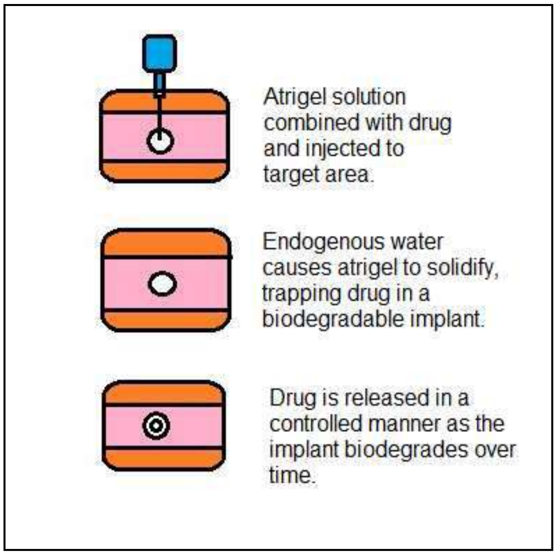
Atrigel solution combined with drug and injected to target area.
Endogenous water causes atrigel to solidify, trapping drug in a biodegradable implant.
Drug is released in a controlled manner as the implant biodegrades over time.

Figure1. Controlled release by Atrigel system

Both in vitro and in vivo release studies were used to optimize the release characteristics of the formulations. For the in vitro studies, the drug is combined with the polymer solution and small drops of the mixture (about 50 mg) are added to phosphate-buffered saline solution.

The receiving fluid is replaced at selected times with fresh solution, and the removed phosphate buffer saline solution is analyzed for drug concentration using a variety of analytical methods (Rathabone 2003).

Atrigels as drug carrier devices:

The most advanced product using Atrigel as a drug carrier, Eligard, incorporating LHRH agonist Leuprolide acetate (7.5, 22.5 or 30 mg) and PLGA 75/25 dissolved in N-methyl-2-pyrrolidone (NMP) in a 45:55 (m/m) polymer: NMP ratio. The carrier system showed reduction in testosterone levels in dogs for approximately 91 days (Ravivarapu 2000) (Chu 2003)- (Garret 2003).

The in vitro release of doxycycline hyclate from implants formed from three different polymers dissolved in NMP has been studied. The more hydrophobic poly (dl-lactide co- caprolactone) (PLC) showed the slowest release of the drug. The hydrophilic poly (dl-lactide-co-glycolide) (PLG) lead to low initial release of drug followed by a more rapid release once the polymer becomes hydrated. The poly (dl-lactide) (PLA) showed the highest initial burst of drug followed by a sustained release out to 8 days (Dunn 1990) .

Polymer molecular weight is also reported to affect the release of drug e.g. in vitro release of naltrexone base from an implant of an Atrigel formulation containing a 50/50 PLG copolymer in NMP was studied. The higher molecular-weight polymer (IV = 0.73 dL/g) showed highest burst release of drug whereas the more moderate-molecular-weight polymer (IV = 0.35 dL/g) gave an almost zero-order release of drug (Tipton, A.J. december 1991).

It has been reported that protein release kinetics was also influenced by solution thermodynamics, e.g. solvent strength and water miscibility. The study includes NMP, triacetin and ethyl benzoate as ternary phase systems with PLGA and water. NMP exhibited a rapid phase inversion associated with a high drug burst, due to the formation of a porous rubbery gel structure. In contrast, other solvents, such as triacetin and ethyl benzoate, both weak solvents for PLGA, yielded low phase inversion rates, resulting in a slow gelation which reduced the drug burst of proteins significantly. Therefore, solvent type and polymer concentration are the most critical factors determining the drug release under in vitro and possibly also in vivo conditions (Brodbeck 1999), (Graham 1999).

O/O emulsion systems have been fabricated using an internal polymer phase (drug, biodegradable polymer and organic solvent) and peanut oil as external phase claiming the in situ formation of microspheres at the injection site as an approach to reduce the unwanted local irritation potential of in situ precipitating systems (Kranz 2001).

A comparative study using different hydrophilic and hydrophobic solvent systems for injectable gels was recently reported by Cleland. In this study, homogenous solutions of poly (D, Llactide), PLA, with the protein were obtained when benzyl alcohol/benzyl benzoate mixtures were used (J.L. 2001).

FUTURE DEVELOPMENTS:

The current ATRIGEL technology appears to provide efficacious products with significant advantages over other existing delivery systems. However, certain improvements been made to the technology include modifications to lower the initial drug burst; use of new polymers and solvents in long-term drug release and tissue compatibility. If these modifications, if, implemented successfully to the Atrigel technology, these will surely increase its uniqueness and its applicability to a wide variety of drug delivery products.

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PARENTERAL IMPLANTS

Patel Ruchita, Mogal Rajendra

Definition:

Implant is an object or material inserted or grafted into the body for prosthetic, therapeutic, diagnostic, or experimental purposes.

Introduction: (Takahashi M 2004)

Implants are one of the dosage forms used to achieve effective concentrations for a long time. Therefore the base materials for implants are required to be biocompatible. Biodegradable and non-biodegradable polymers are often utilized as a base material. Non-biodegradable polymers have to be taken out surgically after completion of the drug release, resulting in pain and a burden on patients. On the other hand, as biodegradable polymers disappear spontaneously from the body during or after drug release, their implants are superior in lowering the burden on patients. In particular, poly-dl-lactic acid (PLA) and poly(dl- lactic acid-co-glycolic acid) copolymer (PLGA) are clinically available as biocompatible and biodegradable polymers, and have been examined extensively and widely. PLGA and PLA show a prolonged drug release for 1 and 3 months, respectively. Other biodegradable polymer like polyanhydride shows a longer drug release about 1 year. Non bio-degradable polymer includes poly vinyl acetate (PVA) etc. Various types of implants are available for the drug delivery system like for delivery into eye, heart, bone, cochlea etc.

Advantages over other other routes: (Takahashi M 2004)

1) Implant dosage forms are useful for patients having difficulty in taking drugs orally, and it allow the avoidance of frequent dosage by sustained supply.
2) A simple administration such as the injection of a drug solution sometimes causes toxic side effects due to a temporary but excessive drug concentration, but in case of implant this side effect can be minimized.
3) I.V. infusion makes the patients fixed on the bed during the treatment, leading to the lack of the normal activities of daily living, while treatment with implants can relieve the patients from the restriction of normal activities of daily living.
4) In the case of time-dependent drugs, as it is therapeutically essential to maintain the drug concentration above the effective lower limit for a long time, excessive drug concentration is not needed. Implants are one of the dosage forms used to achieve effective concentrations for a long time for such time-dependent types of drugs.

Classification:

1. Controlled drug delivery by diffusion process

a. Polymer membrane permeation-controlled drug delivery using
  i. Nonoporous membrane
  ii. Microporous membrane
  iii. Semipermiable membrane
b. Matrix diffusion controlled drug delivery using
  i. Lipophilic polymers
  ii. Hydrophilic polymers
  iii. Porous polymers
c. Microreservior partition controlled drug delivery using
  i. Hydrophilic reservoir in Lipophilic matrix
  ii. Lipophilic reservoir in lipophilic matrix
d. Membrane matrix hybrid type drug delivery using
  i. Lipophilic membrane with hydrophilic matrix
  ii. Hydrophilic membrane with lipophilic matrix

2. Controlled drug delivery by activation process

A. Osmotic pressure activated drug delivery

B. Vapor pressure activated drug delivery

C. Magnetically activated drug delivery

D. Phonophoresis activated drug delivery

E. Hydration activated drug delivery

F. Hydrolysis activated drug delivery

3. Controlled drug delivery by feedback regulated process

I. Bioerosion regulated drug deliver

II. Bioresponsive drug delivery

1. Controlled drug delivery by diffusion process:

a) Polymer membrane permeation controlled drug delivery device:

In this implantable drug delivery device the drug reservoir is encapsulated by a rate controlling polymeric membrane. Different shapes and sizes of implantable drug delivery devices can be fabricated. An example of this type of implantable drug delivery device is the **Norplant** subdermal implant.

Fig:1 Norplant subdermal implant.

b) Polymer matrix diffusion-controlled drug delivery devices:

In this implantable controlled-release drug delivery devices the drug reservoir is formed by homogeneous dispersion of solid particle throughout the a lipophilic or hydrophilic polymer matrix .The dispersion of drug solid particle in the polymer matrix can be accomplished by blending drug solid with a viscous liquid polymer at room temperature followed by crosslinking of polymer chains or by mixing drug solid with a melted polymer dispersion are then molded or excruded toform a drug delivery device of various shapes and sizes. An example of this type of implantable drug delivery device is the **compudose** implant.

Fig: 2 Compudose implant.

c) Membrane-matrix hybrid-type Drug Delivery Devices:

This type of implantable controlled release drug delivery devices is hybrid of the polymer membrane permeation controlled drug delivery system and polymer matrix diffusion- controlled drug delivery system. It aims to take advantages of the constant drug release kinetics maintained by the membrain permeation-drug delivery system while minimizing the risk of dose dumping from the reservoir compartment of this type of drug delivery system.

An example of type of implantable drug delivery device is **Norplant II** subdermal implant.

d) Microreservoir partition-controlled drug delivery devices

In this implantable controlled drug delivery devices the drug reservoir , which is a suspension of drug crystals in an aqueous solution of a water-miscible polymers ,forms a homogeneous dispersion of millions of discrete ,unleachable , microscopic drug reservoir in a polymer matrix.

Different shapes and sizes of drug delivery system by molding or extrusion. Depending upon the physicochemical properties of drug and desired properties of drug rate release, the device can be further coated with a layer of biocompatible polymer to modify the mechanism and rate of drug release.

An example of this type implantable drug delivery device is the **Synchro-Mate** implant. It contains drug norgestomet.

2. Controlled drug delivery by activation process

A) Osmotic pressure-activated drug delivery device

In this implantable controlled-release drug delivery device osmotic pressure is used as the energy source to activate and modulate the delivery of drugs, the drug reservoir, which is either a solution or a semisolid formulation, is contained within is either a solution a semipermiable housing with controlled water permeability.

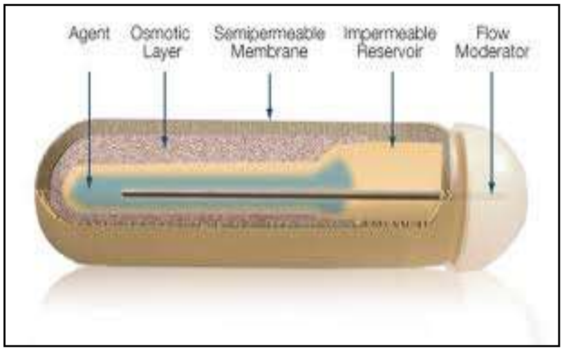

Fig: 3 Alzet osmotic pump.

The physical or chemical properties of a compound have no influence on the delivery rate of ALZET pumps. The delivery rate of ALZET pumps is controlled by the water permeability of the outer membrane. In short, water from the environment enters the pump through the semipermeable membrane into the osmotic layer, which causes compression of the flexible, impermeable reservoir. The test solution is continuously released through the flow moderator. A floe modulator is a hollow tube with an inner diameter of 500 microns. Solutions and even high molecular weight compounds can effectively flow through the flow moderator of ALZET pumps.

Drugs of various molecular configurations, including antibodies, hormones, liposomes, or steroids can be successfully delivered at controlled rate.

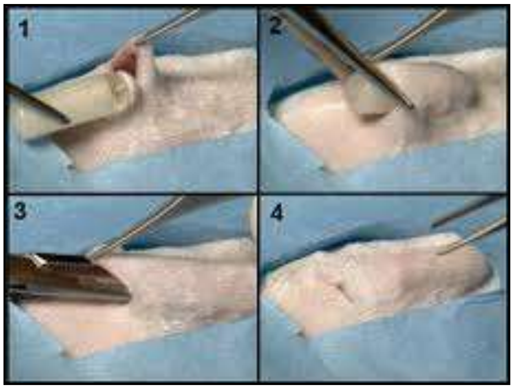

Fig: 4 Administration of Alzet pump.

B) Vapor pressure activated drug delivery devices

In this implantable controlled release drug delivery device vapor pressure is used as the power source to activate the controlled delivery of drugs. The drug reservoir, which is a solution formulation, is contained inside an infusate chamber. By a freely movable bellows the infusate chamber is physically separated from the vapor pressure chamber, which contains the vaporizable fluid, such as fluorocarbon. The fluorocarbon vaporizes at body temperature and creates a vapor pressure that pushes the bellows to move upward and forces the drug solution in the infusate chamber to deliver the drug.

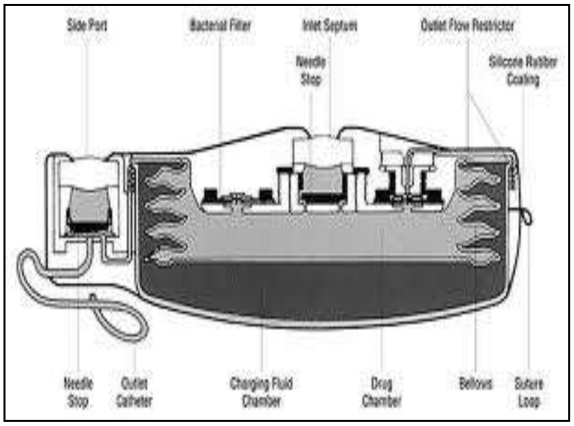

Fig: 5 Infusaid chamber

A typical example of drugs that can be given by this type of infusion pump are **morphine** for patient suffering from intensive pain of terminal cancer, **heparin** for anticoagulation treatment and **insulin** for the treatment of diabetes.

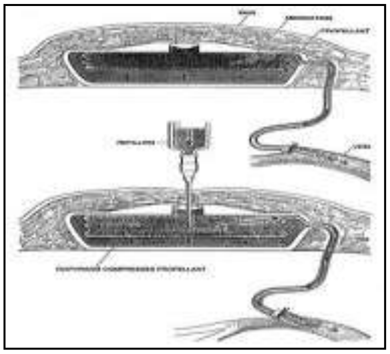

Fig: 6 Administration of Infusaid implant.

C) Magnetically activated drug delivery devices

In this implantable controlled release drug delivery device electromagnetic energy is used as the power source to control the rate of grug delivery. A magnetic wave triggering mechanism is incorporated into the drug delivery device, and drug can be triggered to release at varying rates depending upon the magnitude and the duration of electromagnetic energy applied. This subdermally implantable, magnetically modulated hemispherical drug delivery device was fabricated by positioning a tiny donut-shaped magnet at the center of a medicated polymer matrix that contains a homogenous dispersion of a drug with polymer. The external surface of the hemispherical pellet is further coated with a pure polymer, such as ethylene vinyl acetate copolymer or silicon elastomers, on all sides, expect one cavity at the center of the flat surface, which is left uncoated to permit the drug molecules to be delivered through the cavity.Under nontriggering conditions this implantable hemispherical magnetic pellet releases drugs at a controlled basal rate by diffusion alone. By applying an external magnetic field the drugs are activated by the electromagnetic energy to release from the pellet at a much higher rate of delivery.

D**) Hydration activated drug delivery devices**

This type of implantable controlled release drug delivery device releases drug molecules upon activation by hydration of the drug delivery device by tissue fluid at the implantation site. To achieve this drug delivery device is often fabricated from a hydrophilic polymer that becomes swollen upon hydration. Drug molecules are released by diffusing through the polymer matrix. The hydration activated implantable drug delivery device is exemplified by the development of the norgestomet releasing Hydron implant for estrus synchronization in heifers. This was fabricated by polymerizing ethylene glycol methacrylate (Hydron S) in an alcoholic solution that contains norgestomate, a cross-linking agent(such as ethylene dimethacrylate), and an oxidizing catalyst to form a cylindrical water swellable (but insoluble) Hydron implant.

E) Hydrolysis activated drug delivery devices

This type of implantable controlled release drug delivery device is activated to release drug molecules upon the hydrolysis of the polymer base by tissue fluid at the implantation site. To achieve this the drug delivery device is fabricated by depressing a loading dose of solid drug, in micronized form, homogeneously through a polymer matrix made from bioerodible or biodegradable polymer, which is then molded into a pellet or bead-shaped implant. The controlled release of the embedded drug particles is made possible by the combination of polymer erosion by hydrolysis and diffusion through the polymer matrix. The rate of drug release is determined by the rate of biodegradation, polymer composition and molecular weight, drug loading, and drug-polymer interaction.

The rate of drug release from this type of drug delivery system is not constant and is highly dependent upon the erosion process of the polymer matrix. It is exemplified by the development of biodegradable naltrexone pellets fabricated from poly(lactide-glycolide) copolymer for the antinarcotic treatment of opioid dependent addicts.

In addition to poly(lactide-glycolide) copolymer, several other biodegradable or bioerodible polymers such as polysaccharides, polypeptides and homopolymer of polylactide or polyglycolide, polyanhydride and polycapolactam can also be used.

Implants for eye: (Lee S. S. 2010) (YAHYA E. CHOO PILLAY V. 2009)

The safety and effectiveness of systemic and topical medical therapies for ocular disorders are limited due to poor ocular drug uptake, nonspecificity to target tissues, systemic side effects, and poor adherence to therapy. Intravitreal injections can enhance ocular drug delivery, but the need for frequent retreatment and potential injection-related side effects limit the utility of this technique. Sustained-release drug delivery systems have been developed to overcome these limitations; such systems can achieve prolonged therapeutic drug concentrations in ocular target tissues while limiting systemic exposure and side effects and improving patient adherence to therapy. Topical drug therapy is the primary form of treatment for front-of-the-eye diseases, such as ocular surface diseases (e.g. conjunctivitis, dry eye), for elevated intraocular pressure, and for anterior uveitis. Anatomical and physiological barriers in the eye, including the corneal epithelium and conjunctival clearance mechanisms, afford protection against the entry of xenobiotics. These barriers also greatly impede the entry of drugs to the posterior segment, making it difficult to achieve therapeutic drug concentrations. Treatment of back-of-the-eye diseases such as diabetic retinopathy, neovascular age-related macular degeneration, and retinal venous occlusive disease is especially challenging with topical therapy given the greater diffusional distance. Systemically administered drugs can be used for treating front- and back-of-the-eye diseases. However, the accessibility of ocular tissues is greatly limited by the blood-aqueous and blood-retinal barriers. As a result, high systemic doses must be administered, which increases drug exposure in non-ocular tissues and, consequently, the risk of adverse systemic side effects. Therefore Sustained-release intrascleral and intravitreal drug implants and inserts have been developed for the treatment of ocular diseases.

EXAMPLES OF BIODEGRADABLE OCULAR DRUG DELIVERY SYSTEMS

1) Lacrisert®

It is a sterile, translucent, rod-shaped, water-soluble, biodegradable ophthalmic insert made of hydroxypropyl cellulose (HPC), a physiologically inert substance, for daily administration into the inferior cul-de-sac of the eye. The sustained release of HPC (5 mg) stabilizes and thickens the precorneal tear film and prolongs tear breakup time. The implant is approved to relieve the signs and symptoms of moderate to severe dry eye syndrome, including keratitis sicca, and is indicated especially for patients who remain symptomatic after therapy with artificial tears. Once-daily treatment with Lacrisert was shown to be more effective in relieving dry eye symptoms than 4-times-daily treatments with topical artificial tears.

2) Surodex™

It is a rod-shaped biodegradable matrix implant (1.0×0.5 mm) consisting of dexamethasone and PLGA with hydroxypropyl methylcellulose (HPMC) which provides sustained drug release at a constant rate (60 μg over 7–10 days).

3) Ozurdex

It is a biodegradable implant consisting of 0.7 mg dexamethasone within a solid, rod-shaped PLGA copolymer matrix.The implant is designed to release dexamethasone biphasically, with peak doses for the initial 2 months, followed by lower therapeutic doses for up to 6 months.

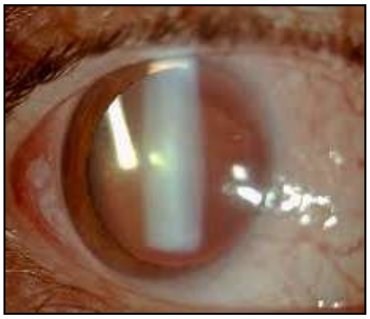

Fig: 7 Ozurdex implant in eye.

NONBIODEGRADABLE OCULAR DRUG DELIVERY SYSTEMS

In nonbiodegradable reservoir-type devices, PVA, a permeable polymer, is typically used as a structural element, while the device’s drug-restricting membrane is composed of EVA, a hydrophobic polymer that is relatively impermeable to hydrophilic drugs.

Examples of Non-biodegradable Ocular Drug Delivery Systems

1) An Ethylene Vinyl Acetate and Poly(Vinyl) Alcohol Reservoir Device (Vitrasert)

Nonbiodegradable polymers were first used clinically for intraocular sustained release of ganciclovir in the

Treatment of cytomegalovirus retinitis (CMV-R). The Vitrasert reservoir-type device composed of drug and polymeric coats of polyvinyl alcohol (PVA) and ethylene vinyl acetate (EVA). The device is implanted in patients. PVA, a permeable polymer regulates the rate of ganciclovir permeation through the device. EVA, an impermeable polymer, limits the surface area of the device through which ganciclovir can be released.

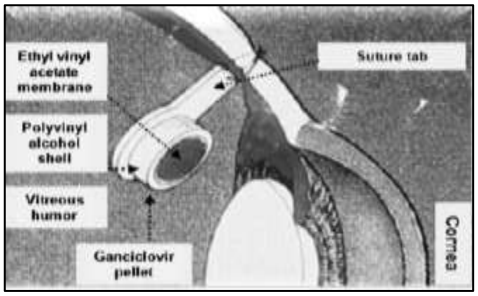

Fig: 8 Vitrasert implant.

2) The Retisert and Medidur Devices

Retisert is a reservoir-based fluocinolone-loaded implant designed to provide drug release over a period of approximately 1000 days. The implant contains 0.59 mg of drug, and it delivers 0.5 mg/day of the corticosteroid, fluocinolone. It is a reservoir-type nonbiodegradable implant.

The Medidur device also contains fluocinolone, but it is a much smaller device.

Fig: 9 Retisert and Medidure implant.

Implants for bone: (Soriano I. 2000) (C.S. Young □ 2009) (Castro C. 2003) (A. Delgadob 2002) (Sanchez E. 2001) (C. Castro 2005)

Orthopaedic surgery carries the risk of osteomyelitis. In general, three approaches are used to prevent or treat post operative bone infections:

1. Prolonged systemic antibiotic therapy for 4±6 weeks.
2. Local implantation of non-degradable antibiotic carriers (like polymethyl methacrylate (PMMA)).
3. Local implantation of non-degradable antibiotic carriers (like PLA, PGA, PLGA).

In systemic therapy, only a small fraction of the dose reaches the surgical site, producing low therapeutic tissue levels and often various adverse systemic side-effects including developement of bacterial resistance to drug. Therefore local antibiotic administration is more convenient.

Many reports have appeared in the literature showing that ceramic materials of beta-tricalcium phosphate (b-TCP) and hydroxyapatite (HAP) can be used in implantable drug delivery systems for local antibiotic treatment of bone infections, since these implants provide bone antibiotic levels with reduction of bacteria levels for a long period of time. Having a chemical composition very similar to the mineral phase of bone, calcium phosphates have been applied clinically as a bone substitute due to its osteointegrative properties and also appears to be well tolerated by the bone tissue, being not only biocompatible but also osteointegrative material.

Ex. Implant composed of **calcium phosphate** cement, **gentamicine** or **ciprofloxacine** and **PLA** that prevent progression of osteomyelitis and induce local bone formation.

Implant after microfracture surgery (In Kyong Shim a 2008)

Articular cartilage has a limited capacity for self repair; particularly of lesions Abrasion arthroplasty and drilling have been used in an attempt to improve the self-regenerative capabilities of cartilage by penetrating the subchondral bone to promote the formation of new in defective cartilage. In particular, microfracture therapy is becoming more widespread since it can be readily performed arthroscopically and achieves good results that last for several years. Microfracture therapy involves using a surgical pick to make holes of approximately 1 mm in diameter in the subchondral bone, resulting in punctuate bleeding and access of blood and marrow to the defect. Following microfracture therapy, new Cartilage tissue is formed. Dehydroepiandrosterone sulfate (DHEA-S) is known to protect against articular cartilage loss. DHEA-S could improve cartilage regeneration with high efficacy after microfracture therapy.

Ex.DHEV-S implantable polymeric rods made of biodegradable poly (D, L-lactide-co-glycolide) (PLGA) for the controlled release of DHEA-S. The implantable polymeric rod was designed to be easily inserted into the holes produced by microfracture surgery.

Implants for heart: (Bege N. 2012)

Percutaneous transluminal angioplasty (PTCA) is used in the treatment of Coronary Artery Disease. Over the past decade, extensive research has been performed addressing the design of stents, which are commonly used for PTCA. Endoluminal metallic endoprostheses (stents) have reduced procedural complications in PTCA like elastic recoil of the vessel wall, balloon-induced dissection, and reoccurrence of restenosis. To overcome the restenosis issue, stents for local delivery of several drugs were established. First generation drug eluting stents (1GDES) consist of a backbone stent (316 L stainless steel or Nitinol), a polymer (biodegradable or non-degradable), and drugs such as Paclitaxel or Sirolimus. This 1 G-DES were designed to reduce in stent neointimal formation and to minimize the appearance of restenosis.

CYPHER Stent: A stent is a permanent implant that remains in your artery. CYPHER® Stent is a small, expandable, slotted metal tube is inserted through a catheter into a coronary artery. There, it acts as a scaffold to help hold the artery open in order to improve blood flow to the heart and relieve the symptoms and dangers associated with artery blockage. The CYPHER® Stent is a drug-eluting stent. The metal of the stent has a soft, plastic coating that contains the anti-rejection-type medicine sirolimus. It slowly releases this medicine into the artery wall around the stent to help limit the normal overgrowth of tissue as the healing process takes place. Eighty percent (80%) of the sirolimus is released during the first 30 days. The rest is released by the end of 90 days.

TAXUS stent: The TAXUS stent uses Translute™ Polymer, a proprietary polymer carrier technology, to control drug release. The durable Translute Polymer protects the drug and maintains coating integrity during preparation, delivery, and stent expansion. The polymer controls the release of paclitaxel, which may allow for consistent drug release and more uniform drug distribution.

Fig: 10 Metallic stent.

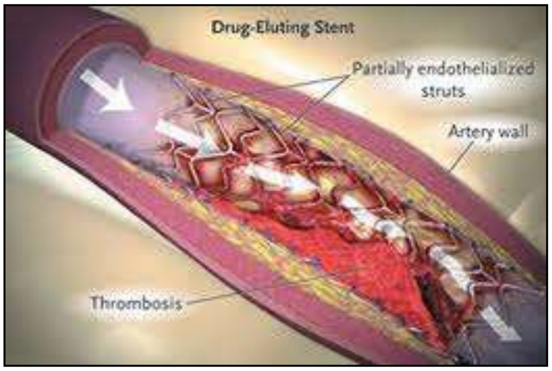

Fig: 11 Drug loaded stent inserted into coronary artery.

***In-situ* implant:** (Patel R. B. 2010)

The development of in situ forming implants (ISFIs) has been a topic of significant research interest in recent years. The term ISFI has been used to describe a variety of polymer based systems that are initially comprised of a liquid solution that undergoes a process of solidification after placement in a site of interest. In-situ implant formed by a biodegradable polymer dissolved in water miscible organic solvent that undergoes a process of liquid de-mixing when injected into a water rich phase. Upon injection into the aqueous environment, the water-insoluble polymer component of the ISFI solution precipitates as the water miscible organic solvent leaches out into the bath solution and water diffuses into the implant, a process commonly referred to as phase inversion.

The ISFI systems have several **advantages** compared to traditional pre-formed implant systems. Due to their injectable nature, implant placement is less invasive and painful for the patient thereby improving comfort and compliance. Additionally the manufacturing process required for fabrication is relatively mild and straightforward making ISFI systems particularly suitable for delivery of fragile protein and peptide therapies.

Currently, only two FDA approved products are on the market utilizing this type of system, **Eligard®** and **Atridox®**. Eligard®, using the Atrigel® delivery system is a subcutaneously injected implant that releases leuprolide acetate over a period of 3 months to suppress testosterone levels for prostate cancer treatment.

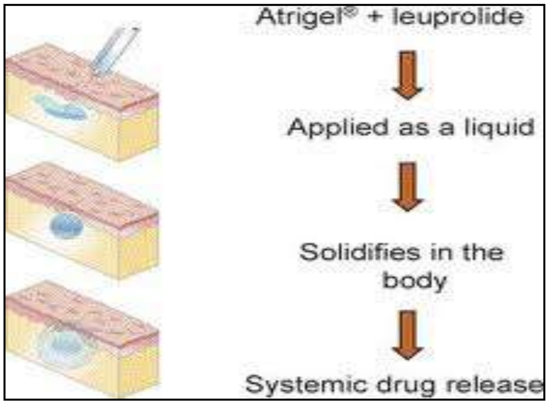

Fig: 12 Eligard *in-situ* implant

Atridox® is another ISFI system that also uses the Atrigel® delivery system to deliver the antibiotic agent, doxycycline, to the sub-gingival space to treat periodontal disease.

Fig: 13 Atridox *in-situ* implant

Lipid implant:
Lipid implant shows release rates are generally lower than those of implants prepared by direct the resulting drug release rates are generally lower than those of implants prepared by direct compression.

Advantages of lipid implant over biodegradable polymer implant:

1) No acidic microclimates are created within the systems, in contrast to the widely used poly(lactic-co-glycolic acid) (PLGA) based devices. Such acidic microenvironments can cause the inactivation of incorporated drugs, especially of acid-labile proteins.

2) In addition, no organic solvents are required during the manufacturing of lipid implants. Thus, there is no risk of drug denaturation at water/solvent interfaces and no risk of solvent toxicity (neither for the patient nor for the environment).

Hence, lipid implants present an interesting alternative to PLGA-devices for controlled parenteral drug delivery. Proteins can be delivered by lipid implant.

Ex. Implant consist of a **lipid component**, **protein** (like rh-INFα), **HP-β-CD**(Hydroxy propyl β cyclodextrin) or **trehalose** as stabilizer and **PEG-6000** as a release modifier. The controlled release is due to the interaction of PEG with the incorporated rh-INFα, leading to reversible precipitation of the protein within the matrix, resulting the dissolution the INFα contributes to the controlled release.

Cochlear implant: (Krenzlin S. 2012)

Miniaturized implants releasing active agents in a time-controlled manner offer a great potential, especially in the case of local drug treatments. For instance, drug delivery to the inner ear is highly challenging due to the blood-cochlear barrier, which is anatomically and functionally similar to the blood-brain-barrier. Upon drug administration via common routes (including oral, i.v., i.m. etc.) often only minor amounts of the active agent reach the target site (e.g. hair cells, lateral cochlear wall), because tight junctions effectively hinder the passage from the systemic circulation into the inner ear. This might be compensated by high drug doses. However, high systemic drug concentrations might also lead to severe side effects. To overcome these restrictions the drug might be directly administered into the inner ear. However, since the cochlea is a very small, closed space and sensitive to minor changes in fluid volume, such direct administrations are highly delicate. Furthermore, if a drug solution is injected, the active agent might be rapidly eliminated from the inner ear, partially due to efflux through the injection channel (note that this is not true for all drugs). Thus, frequent injections might be required in these cases. This is not feasible due to the risk of infections and continued alterations in the volume of the perilymph (which is the major bulk fluid in the cochlea). To overcome these hurdles and to allow for local drug treatment of the inner ear, different types of time-controlled delivery systems have been proposed. For instance, semi-solid hydrogels have been described as matrix formers for administration onto the round window. However, the residence times of these systems at this location can significantly vary and there is a considerable uncertainty with respect to the amount of drug that reaches the inner ear and the time period during which drug is delivered. Also in the case of solid, miniaturized implants releasing the drug through a small hole drilled into this membrane, the residence time is uncertain, since they cannot be tightly and securely fixed in vivo. Furthermore, the perilymph can be considered as a virtually non-agitated fluid with negligible convective mass transport. Thus, if the device is administered into the middle ear, the resulting drug concentration in the perilymph might not be homogeneous (e.g., high concentration close to the round window, which steeply decrease along the scala tympani. Implants with the geometry of long, very thin cylinders (like thin “spaghetti”), which are placed directly into the scala tympani of the cochlea, can offer an interesting potential to overcome all these restrictions.

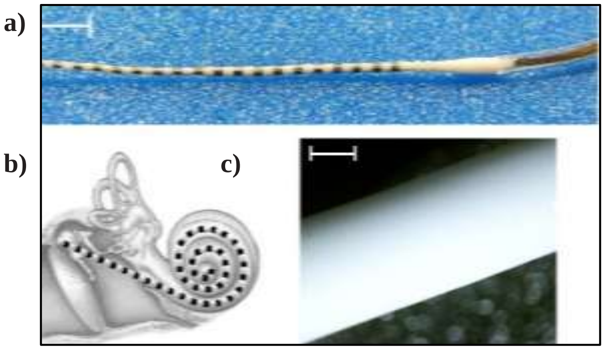

Fig: 14 (a) macroscopic image of a cochlear implant loaded with 10% dexamethasone. (b) Schematic presentation of the administration site of the cochlear implant . (c) Macroscopic image of a cylindrical extrudate loaded with 10% dexamethasone.

Preparation method for implant:

1**) Direct compression method:**

The implant components are ground in mortar and resulting mixture is compressed.

2) **Solvent evaporation:** (Shyam S. Bansal a 2012)

In this method the drug is dissolved in ethanol, and polymers are dissolved in dichloromethane. Both solutions are then mixed together to prepare a homogenous solution of drug and polymers. The solvents are evaporated on a waterbath maintained at 65^{o}C followed by overnight drying under vacuum at 65^{o}C to remove residual solvents to prepare an amorphous molecular dispersion of drug in polymer. The dried molten polymer is then extruded through silastic tubing mold (internal diameter 3.4 mm) attached to a syringe. After few min, the cylindrical implants are removed from the tubing and excised into desired lengths.

Preparation method for lipid implant: (Kreye F. 2011)

The drug: lipid powder blends generally show poor flowability. For example, de-mixing can lead to inhomogeneous drug contents. These restrictions can be overcome when using a different implant preparation technique: melting and casting. The idea is to heat the lipid (which generally exhibits a relatively low melting temperature), homogeneously distribute the drug(s) in the melt, and to cast the solutions/suspensions into molds of arbitrary geometry and size. Ideally, the device dimensions should allow for easy administration, e.g., tiny cylinders which can be injected using conventional needles. Obviously, the incorporated drug must be stable at the applied production temperatures. It has to be pointed out that even many proteins are stable at elevated temperatures, if water is absent.

In-vitro characterization parameters for implant: (Kreye F. 2011) (Takahashi M. 2004)

1) *In-vitro* release studies

Implants are placed into tubes, which is filled with phosphate buffer pH 7.4 (USP 33) and horizontally shaken at 37 °C (80 rpm) (1 implant per tube). At predetermined time points, the release medium is completely replaced with fresh phosphate buffer, and the drug content in the withdrawn bulk fluid is measured by UV-spectrophotometry.

2) Drug content

An implant system is dissolved in a mixture of dichloromethane and methanol (1:1, v/v), and the solution is measured spectrophotometrically at the λ_{max} of the drug to determine the concentration of drug using UV spectrophotometer.

3) Water uptake and erosion studies

Implants are placed into phosphate buffer pH 7.4. At predetermined time points, implants are withdrawn, excess surface water carefully removed, the systems accurately weighed [wet mass (t)] and dried to constant weight in an oven at 37 °C [dry mass (t)]. The water content (%) (t) and lipid matrix erosion (%) (t) Were calculated as follows:

Water content (%)(t) = wet mass- Dry mass/ Wet mass × 100

Lipid matrix erosion = Dry mass(0) – Drug released(t) – Dry mass(t) /Dry mass(0) ×100%

Where ‘‘dry mass (0)’’ denotes the dry implant mass at t = 0 and ‘‘drug released (t)’’ the cumulative amount of drug released at time t.

4) Thermal analysis

The thermal properties of lipid powders and implants are determined by differential scanning calorimetry (DSC). Temperature and enthalpy readings are calibrated with pure indium. Approximately 2–3 mg samples were heated in sealed aluminum pans [temperature range: 20–90 °C; heating rate: 5 °C/min].

5) Hardness of implantable tablet

The flat surface of the implant tablet is set on a flat plate with a ditch (5 mm width) in the center, which is set parallel to the horizontal surface. A spherical iron ball (5 mm diameter), attached to the rheometer is pressed against the flat surface of the tablet at a speed of 6 cm/min. The stress (g) observed, immediately before the implant tablet is broken, is measured as the hardness of the tablet.

For *in-situ* implant evaluation: (Kempe S. 2010)

1) *In-vitro* EPR (electron paramagnetic resonance) spectroscopy

For in vitro monitoring of the in situ forming implant formation. The polymer solutions were injected into perforated plastic capsules placed in the buffer solutions, where the implants formed immediately. At determined time points the implants were taken out the buffer, the capsules were dried with paper, wrapped into plastic foil to prevent drying and subsequently transferred to the EPR spectrometer. The EPR measurements were performed by an L-band spectrometer.

2) *In-vivo* BT-MRI (bench top magnetic resonance imaging) study

The polymer solution or PEG 400, as control, was injected in to the lower back of the mice. The mice were anesthetized prior to injection and each measurement by inhalation of isoflurane. MRI measurements were carried out at regular intervals after every 10 min within the first hour after injection and then after 2 h, 3 h, 6 h and 24 h. The subsequent measurements were performed once a week up to 2 months.

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TRANSDERMAL DRUG DELIVERY SYSTEM

Pawar Sandesh, Katedeshmukh Ramesh

Introduction:

Transdermal drug delivery offers many potential advantages **over conventional methods, such as pills and** injections. Administration across skin avoids gastrointestinal and first-pass liver degradation associated with oral delivery, provides a user-friendly approach, and can be formulated to deliver a drug in a continuous or controlled manner. However, most drugs diffuse across skin too slowly for medical applications. The skin's impressive barrier properties are due primarily to the outer 10–15 mm, called stratum corneum, which contains keratin-filled cells surrounded by an extracellular milieu of lipid organized in multilamellar bilayer structures. It is these stacks of intercellular lipid bilayers which limit transdermal transport and must be overcome when delivering drugs across skin. To increase rates of transdermal delivery a number of different enhancement methods have been studied, including chemicals, liposomes, ultrasound, microneedles and electricity. The mechanism by which these enhancers work involve one or both of the following two aspects: (1) changes in the skin's chemical and/or physical environment which allow more drug to penetrate into the stratum corneum and (2) application of a driving force which induces transport across the stratum corneum. Chemical enhancers [5] can alter the chemical and physical environment within the skin so that drug more effectively partitions into the tissue. This involves increasing drug solubility in the stratum corneum and/or disrupting lipid structure. The mechanism of action for liposomes remains somewhat unclear, but probably involves enhanced partitioning into the skin by changing the local chemical environment. Ultrasound has been used both at high frequency , where enhancement is largely due to a pressure-driven driving force possibly coupled with physical disruption of lipids, and at low frequency , where ultrasound-induced cavitation causes extensive physical disruption of stratum corneum microstructure and also provides a pressure-driven driving force. Recently, arrays of micron-sized microneedles which cross stratum corneum but do not stimulate nerves in deeper tisue,havebeen shown to create physical conduits for transport across skin. Finally, electricity has been used both at low voltage , where iontophoresis drives molecules across the skin and secondarily induces physical changes in skin microstructure, and at high voltage , where electroporation both rearranges the structure of stratum corneum's lipid bilayers and provides an electrophoretic driving force. This final method involving electroporation of skin using high-voltage pulses is the subject of this review for transdermal delivery of small, lipophilic drugs which are effective at low doses, no enhancement methods are needed. For other small compounds (e.g., more hydrophilic and/or those requiring larger doses), methods which provide an added driving force (e.g.,iontophoresis, high-frequency ultrasound) or make changes in the skin's chemical environment (e.g., chemical enhancers, liposomes) have been shown to be helpful. However, for larger compounds (e.g., macromolecules) or those with especially challenging delivery requirements (e.g., large se, pulsed or variable delivery), methods which physically disrupt the skin (e.g., electroporation, low-frequency ultrasound, microneedles) are needed . This review summarizes the evidence which shows how electroporation physically disrupts the skin and assesses the prospects for its application to transdermal drug delivery (R.Prausnitz 1995).

Mechanism of Drug Delivery in Transdermal Drug Delivery System:

A transdermal drug delivery system is a device that is made of one or more types of polymers embedded with drug(s) to deliver the embedded drug through the skin over a controlled period of time. Figure 1 shows the schematic of a typical transdermal drug delivery system with the patch and its different layers involved. The concept of transdermal patch was introduced in 1979 and the patch was scopolamine patch (4) that delivers a drug which is used for motion sickness. The principle mechanism for drug delivery in transdermal drug delivery system is “a slow process of diffusion driven by the gradient between the high concentration in the delivery system and the zero concentration prevailing in the skin”.The drug permeation across the skin obeys Fick’s first law where steady-state flux (J) is related to the diffusion coefficient (D) of the drug in the stratum corneum over a diffusional path Length or membrane thickness (h), the partial coefficient (P) between the stratum cornuem and the vehicle, and the applied drug concentration (C_0) which is assumed to be constant”

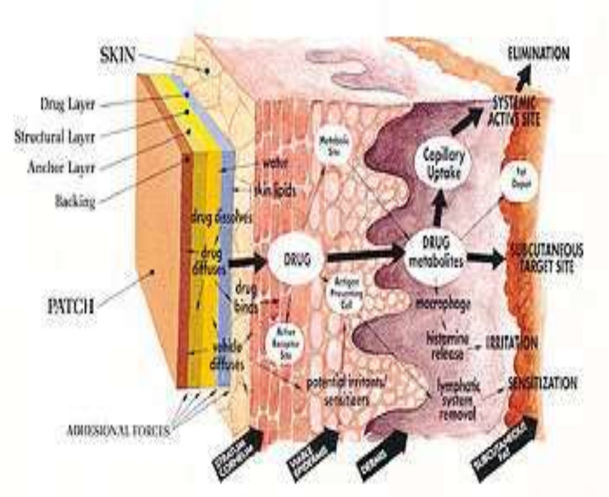

Figure 1: Schematic Diagram of transdermal delivery system

The mechanism of drug delivery in a transdermal patch is diffusion through skin and skin poses a barrier. The original function of the skin is to give out things that are not required in the body, for instance, sweat, dirt etc. It also maintains the temperature of the body. The major obstruction is posed by the top most epidermal layer of stratum corneum.

The stratum corneum is the outermost layer of the skin, which is considered to be dead skin. It has the undesirable properties as follows:

- Hygroscopic, yet impermeable to water
- Tough membrane
- Flexible membrane
- Intercellular space rich in lipids
- Thickness varies for different parts of the body

Even though the stratum corneum has the above mentioned undesirable properties, it allows permeation of some chemicals to reach onto the tissue and the blood vessels underneath the skin. The chemical also require special formulation characterized by low molecular weight, lipophilicity, and being effective even at low dosage levels.The formulation is still effectively kept and achieved by keeping the patch for several hours a day or up to several days a week. Butthere are only a handful of drugs that can be employed keeping low molecular weight and low dosage. This obstruction of the stratum corneum is overcome by use of other penetration enhancer techniques like use of a mechanical array on the Transdermal drug delivery patch (D.Patel 2011)

Advantages of transdermal drug delivery systems

Delivery via the transdermal route is an interesting option because transdermal route is convenient and safe. The positive features of delivery drugs across the skin to achieve systemic effects are:

- Avoidance of first pass metabolism
- Avoidance of gastro intestinal incompatibility
- Predictable and extended duration of activity

- Minimizing undesirable side effects
- Provides utilization of drugs with short biological half lives, narrow
- Therapeutic window
- Improving physiological and pharmacological response
- Avoiding the fluctuation in drug levels
- Inter and intra patient variations
- Maintain plasma concentration of potent drugs
- Termination of therapy is easy at any point of time
- Greater patient compliance due to elimination of multiple dosing profile
- Ability to deliver drug more selectively to a specific site
- Provide suitability for self administration
- Enhance therapeutic efficacy (D.Patel 2011)

Polymers in Transdermal Drug Delivery System:

As described in the section on the FDA Regulation and Transdermal Drug Delivery system,Transdermal drug delivery system is a combinational product i.e., it is a combination of a drug and a device to administer the drug into the biological system. “The primary jurisdiction for combination products (e.g., device, drug, or biologic) is determined by the primary mode ofaction of the product”. In the case of a transdermal drug delivery system, the primary mode of action of the product is controlled drug release and hence it becomes extremely crucial to understand and select materials and techniques that would make it possible to control the drugrelease. Controlled delivery of drug from the device to the biological system can be achieved byvarious means. An IV fluid drip also controls the rate of flow of drug, but the apparatus is bulkyand cannot be used on a daily basis for home care patients. Controlled drug releases can also beachieved by embedding the drug onto a polymeric material and then releasing the drug in a predesigned controlled manner from the polymer into the blood stream. This is achieved by these of transdermal drug delivery system that is made of polymeric materials. it gives a list of polymers that are extensively used in making transdermal patches. It becomes evident from that there are various types of polymers that are used for different types of transdermal drug delivery systems. There are some polymers that are used as adhesives, some as backing layer for the transdermal patch, some to create a gel that would help embed the drug for the controlled delivery of the drug. Every layer in the transdermal drug delivery system requires properties specific for that layer only. The type of polymer is chosen according to the desired properties for those specific layer.The physical properties and the polymers showing those properties are listed below:

- Poly (urethanes) for elasticity
- Poly (siloxanes) or silicones for insulating ability
- Poly (methyl methacrylate) for physical strength and transparency
- Poly (vinyl alcohol) for hydrophilicity and strength
- Poly (ethylene) for toughness and lack of swelling
- Poly (vinyl pyrolidone) for suspension capabilities

The other types of polymers are those that are desired for the controlled release of the drug.

The polymers that are currently used in the manufacturing of the transdermal drug delivery systems are Poly(2-hydroxy ethyl methacrylate), Poly(N-vinyl pyrrolidone), Poly(methyl methacrylate) better known as PMMA, Poly(vinyl alcohol), Poly(acrylic acid), Polyacrylamide, Poly(ethylene-co-vinyl acetate), Poly(ethylene glycol), Poly(methacrylic acid).

The desired type of controlled drug delivery mechanism by the above mentioned polymers are shown schematically in **Figure 3**

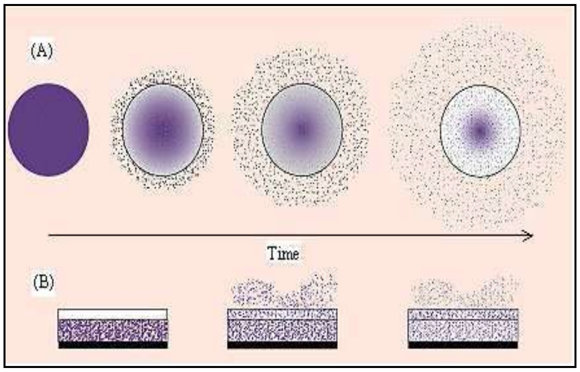

Figure 3: Controlled Drug Release Mechanism
Figure 3**(A)** shows the drug delivery in a matrix type of system while **(B)** shows the reservoir
type of transdermal drug delivery system.

The other property which is of utmost importance is that of biocompatibility. The polymersthat are considered to have properties desirable for transdermal drug delivery system must also be biocompatible since the application of a transdermal patch onto the surface of the skin can before several days. The other aspect of compatibility is that of chemical compatibility, the drug that is to be embedded in the polymer must be chemically compatible and must not react with the polymer. With the active transdermal systems, it also becomes important that techniques and polymeric materials used for penetrating stratum corneum do not react with the drug that is to be administered. The following section takes into account all the factors to be considered and describes as well as differentiates the use of polymers in the transdermal drug delivery system for different components of transdermal drug delivery system. (Donald 2007)

Basic components of transdermal drug delivery systems:

Polymer matrix: Polymer is an integral and foremost important component of transdermal drug delivery systems. Different classes of polymeric materials have been used to achieve rate controlled drug delivery. The mechanism of drug release depends upon the physicochemical properties of the drug and polymer used in the manufacture of the device. The following criteria should be satisfied for a polymer to be used in a transdermal system.

Molecular weight, glass transition temperature, chemical functionality or polymer must allow diffusion and release of the specific drug. The polymer should permit the incorporation of a large amount of drug. The polymer should not react, physically or chemically with the drug. The polymer should be easily manufactured and fabricated into the desired product and in expensive. The polymer must be stable and must not decompose in the presence of drug and other excipients used in the formulation, at high humidity conditions, or at body temperature.

Polymers and its degradation products must be non toxic. No single material may have all these attributes; e.g., cosolvents such as ethanol, propylene glycol, PEG 400 could be added to increase drug solubility (somnath 2005)

Various techniques which are employed to modify the polymer properties and thus drug release rates:

Cross linked polymers: The higher the degree of cross linking, the more dense the polymer and slower the diffusion of drug molecules through the matrix.

Polymer blends: Polymers have been blended on varying ratios to combine the advantages of the individual polymers. Advantages of polymer blends include easy fabrication of devices, manipulation of drug loading and other devices properties such as hydration, degradation rate and mechanical strength.

Plasticizers: Plasticizers have been known to reduce the stiffness of the polymer backbone, thereby increasing the diffusion characteristics of the drug. Commonly used plasticizers are polyethylene glycol, propylene glycol, glycerol, dibutyl phthalate.

Drug substance:

Drug is in direct contact with release liner.

Ex: Nicotine, Methotrexate and Estrogen.

The selection of drug for transdermal drug delivery depends upon various factors.

Physicochemical properties:

1. The drug should have some degree of solubility in both oil and water (ideally greater than 1 mg/ml)

2. The substance should have melting point less than 200 °F. Concentration gradient across the membrane is directly proportional to the log solubility of drug in the lipid phase of membrane, which in turn is directly proportional to the reciprocal of melting point (in degree absolute of the drug). In order to obtain the best candidates for TDD, an attempt should be made to keep the melting point as low as possible.

3. Substances having a molecular weight of less than 1000 units are suitable.

4. A saturated aqueous solution of the drug should have a pH value between 5 and 9. Drugs highly acidic or alkaline in solution are not suitable for TDD; because they get ionized rapidly at physiological pH and ionized materials generally penetrate the skin poorly.

5. Hydrogen bonding groups should be less than 2.

Biological properties:-

Drug should be very potent, i.e., it should be effective in few mgs per day (ideally less than 25 mg/day)
The drug should have short biological half life
The drug should be non irritant and non allergic to human skin The drug should be stable when in contact with the skin The drug should not stimulate an immune reaction to the skin Tolerance to drug must not develop under near zero order release profile of transdermal delivery The drug should not get irreversibly bound in the subcutaneous tissue
The should not get extensively metabolized in the skin

Penetration enhancers:-

These are the compounds, which promote skin permeability by altering the as a barrier to the flux of a desired penetrate and are considered as an integral part of most transdermal formulations. To achieve and maintain therapeutic concentration of drug in the blood, the resistance of skin to diffusion of drugs has to be reduced in order to allow drug molecules to cross skin and to maintain therapeutic levels in blood. They can modify the skin's barrier to penetration either by interacting with the formulation that applied or with the skin itself the penetration enhancer should be pharmacologically inert, non toxic, non allergenic, non-irritating and ability to act specifically, reversibly and for predictable duration. It should not cause loss of body fluids, electrolytes or other endogeneous materials.
Ex: Terpenes, Terpenoids, Pyrrolidones.
Solvents like alcohol, Ethanol, Methanol.
Surfactants like Sodium Lauryl sulfate, Pluronic F127, Pluronic F68.

Drug reservoir components :

It must be compatible with the drug and must allow for drug transport at the desired rate. If an ointment is used, the drug reservoir must possess the desired viscosity attributes to ensure reliable manufacturing process. It must possess the desired adhesive and cohesive properties to hold the system together. Materials used are: mineral oils, polyisobutylene, and colloidal silica, HPC.

Backing laminates:-

The primary function of the backing laminate is to provide support. They should be able to prevent drug from leaving the dosage form through top. They must be impermeable to drugs and permeation enhancers. They should a low moisture vapor transmission rate. They must have optimal elasticity, flexibility, and tensile strength. They must be chemically compatible with the drug, enhancer, adhesive and other excipients. They must be relatively inexpensive and must allow printing and adhesive lamination. Type backing membranes are composed of a pigmented layers, an aluminium vapor coated layer, a plastic film (polyethylene, polyvinyl chloride, polyester) and a heat seal layer.

Rate controlling membrane:-

Rate controlling membranes in transdermal devices govern drug release from the dosage form. Membranes made from natural polymeric material such as chitosan show great promise for use as rate controlling membranes. Recently composite poly-2-hydroxyethyl methacrylate (PHEMA) membranes have been evaluated as rate controlling barriers for transdermal application.

Adhesive layer:

The fasting of all transdermal devices to the skin using a pressure sensitive adhesive that can be positioned on the face or in the back of device is necessary. It should not cause irritation, sensitization or imbalance in the normal skin flora during its contact with the skin. It should adhere to the skin aggressively. The three major classes of polymers evaluated for potential medical applications in TDDS include:

- Polyisobutylene type pressure sensitive adhesives
- Acrylic type pressure sensitive adhesives
- Silicone type pressure sensitive adhesives

Release liners:

The release liner has to be removed before the application of transdermal system, and it prevents the loss of the drug that has migrated into the adhesive layer during storage. It also helps to prevent contamination. It is composed of a base layer, which may be nonocclusive or occlusive, and a release coating layer made of silicon or Teflon. Other materials include polyesters, foil, Mylar and metalized laminate (D.Patel 2011)

Polymers:

Polymers are the backbone of a transdermal drug delivery systemSystems for transdermal delivery are fabricated as multilayeredpolymeric laminates in which a drug reservoir or adrug–polymer matrix is sandwiched between two polymericlayers: an outer impervious backing layer that prevents the lossof drug through the backing surface and an inner polymericlayer that functions as an adhesive and/or rate-controlling membrane.Transdermal drug delivery systems are broadly classifiedinto the following three types (vinod nair 2006)

Reservoir systems:

In this system, the drug reservoir is embedded Between an impervious backing layer and tecontrollingmembrane. The drug releases only throughtherate-controlling membrane, which can be microporous or nonporous.In the drug reservoir compartment, the drug can be in the form of a solution, suspension, or gel or dispersed in asolid polymer matrix. On the outer surface of the polymericmembrane a thin layer of drug-compatible, hypoallergenicadhesive polymer can be applied. (vinod nair 2006)

Matrix systems:

Drug-in-adhesive system. The drug reservoir isformed by dispersing the drug in an adhesivepolymer and thenspreading the medicated polymer adhesive by solvent castingor by melting the adhesive (in the case of hot-melt adhesives)onto an impervious backing layer. On top of the reservoir, layersof unmedicated adhesive polymer are applied. (vinod nair 2006)

Matrix-dispersion system:

The drug is dispersed homogeneouslyin a hydrophilic or lipophilic polymer matrix. This drugcontainingpolymer disk then is fixed onto an occlusive baseplate in a compartment fabricated from a drug-impermeablebacking layer. Instead of applying the adhesive on the face ofthe drug reservoir, it is spread along the circumference to form a strip of adhesive rim (vinod nair 2006)

Microreservoir systems:

This drug delivery system is a combination of reservoir and matrix-dispersion systems. The drug reservoir is formed by first suspending the drug in an aqueoussolution of water-soluble polymer and then dispersing the solutionhomogeneously in a lipophilic polymer to form thousandsof unleachable, microscopic spheres of drug reservoirs.The thermodynamically unstable dispersion is stabilized quicklyby immediately cross-linking the polymer in situ.Transdermal drug delivery technology represents one of themost rapidly advancing areas of novel drug delivery. Thisgrowth is catalyzed by developments in the field of polymerscience. This article focuses on the polymeric materials usedin transdermal delivery systems, with emphasis on the materials' physicochemical and mechanical properties, and it seeksto guide formulators in the selection of polymers.Polymersare used in transdermal delivery systems in various ways, including as

- Matrix formers
- rate-controlling membranes
- pressure-sensitive adhesives (PSAs)
- backing layers
- release liners.

Polymers used in transdermal delivery systems should havebiocompatibility and chemical compatibility with the drug andother components of the system such as penetration enhancersand PSAs. They also should provide consistent, effective deliveryof a drug throughout the product's intended shelf life ordelivery period and have generally-recognized-as-safe status.From an economic point of view, a delivery tool kit rather thana single delivery tool is most effective Companies involved in the field of transdermal delivery concentrateon a few selective polymeric systems. For example, AlzaCorporation (Mountain View, CA) mainly concentrates on ethylenevinyl acetate (EVA) copolymers or microporous polypropylene,and Searle Pharmacia (Barceloneta, PR) concentrates onsilicone rubber . A review of the marketed transdermal productsand theformulations that are reported in various researchpublications reveals an enormous diversity of polymers usedin the formulation, engineering, and manufacture of drug products Table II is a comprehensive list of all the polymersused for various purposes in commercially availabletransdermaldelivery systems. (Roxhed 2007)

Matrix formers:

Polymer selection and design must be considered when strivingto meet the diverse criteria for the fabrication of effectivetransdermal delivery systems. The main challenge is in the designof a polymer matrix, followed by optimization of the drug loaded matrix not only in terms of release properties, but alsowith respect to its adhesion–cohesion balance, physicochemicalproperties, and compatibility and stability with other componentsof the system as well as with skin A monolithic solid-state design often is preferred for passivetransdermal delivery systems because of manufacturing considerationsand cosmetic appeal. Although polymeric matricesare used for rate control, adhesion (e.g., a PSA), or encapsulationof a drug reservoir in transdermal delivery systems (reviewedin later sections of this article), discussion in this sectionis limited to polymers that have been used in the design ofmatrices with or without rate control. (Roxhed 2007)

Cross-linked poly (ethylene glycol) (PEG) networks:

Biocompatibilityof PEGs makes them the polymers of choice for numerousbiomedical applications. Proteins can be delivered by PEGscross-linked with tris (6-isocyanatohexyl) isocyanurate by means of a urethane–allophanate bond to obtain polymernetworkscapable of swelling in phosphate-buffered saline or ethanol andforming gels. These systems have been shown to release the solutes in a biphasic manner. (Roxhed 2007)

Acrylic-acid matrices:

Acrylic-acid matrices with plasticizershave been used to make drug–polymer matrix filmsfortransdermaldelivery systems. Some of the polymers that have beenreported are Eudragit RL PM, Eudragit S-100, Eudragit RS PM, and Eudragit E-100 (Röhm America, Piscataway, NJ).Eudragit NE-40D (a copolymer of ethyl acrylate and methyl Methacrylate), a nonadhesive hydrophobic polymer, also has been used as a matrix former. The release rates of drugsfrom these matrix systems are more closely described by thesquare-root-of-time model. (Roxhed 2007)

Ethyl cellulose (EC) and polyvinylpyrrolidone (PVP)

EC and PVP matrix films with 30% dibutyl phthalate as a plasticizer havebeen fabricated to deliver diltiazem hydrochloride and indomethacin.The addition of hydrophilic components such asPVP to an insoluble film former such as ethyl cellulose tends toenhance its release-rate constants. This outcome can be attributedto the leaching of the soluble component, which leads tothe formation of pores and thus a decrease in the mean diffusionpath length of drug molecules to release into the dissolutionmedium. The result is higher dissolution rates. Substancessuch as PVP act as antinucleating agents that retard the crystallizationof a drug. Thus they play a significant role in improvingthe solubility of a drug in the matrix by sustaining the drugin an amorphous form so that it undergoes rapid solubilizationby penetration of the dissolution medium. (N.E 2004)

Hydroxypropyl methylcellulose (HPMC)

HPMC, a hydrophilicswellable polymer widely used in oral controlled drug delivery, also has been explored as a matrix former in the design ofpatches of propranolol hydrochloride. HPMC has been shownto yield clear films because of the adequate solubility of the drugin the polymer. Matrices of HPMC without rate-controllingmembranes exhibited a burst effect during dissolution testingbecause the polymer was hydrated easily and swelled, leadingto the fast release of the drug (N.E 2004).

Conventional vesicles as skin delivery systems:

General

Vesicles are water-filled colloidal particles. The walls of these capsulesconsist of amphiphilic molecules in a bilayer conformation.In an excess of water these amphiphilic molecules can formone (unilamellar vesicles) or more (multilamellar vesicles)concentric bilayers Hydrophilic drugs can be entrappedinto the internal aqueous compartment, whereas amphiphilic,lipophilic and charged hydrophilic drugs can be associatedwith the vesicle bilayer by hydrophobic and/orelectrostatic interactions A wide variety of lipids and surfactants can be used toprepare vesicles. Most commonly, the vesicles are composedof phospholipids or non-ionic surfactants These are referred to as LIPOSOMES and niosomes or nonionicsurfactant vesicles, respectively. The composition ofthe vesicles influences their physico-chemical characteristicssuch as, size, charge, thermodynamic phase, lamellarityand bilayer elasticity. These physico-chemical characteristicshave a significant effect on the behaviour of thevesicles and hence on their effectiveness as a drug deliverysystem.The rationale for using vesicles in dermal and transdermaldrug delivery is many foldsVesicles might act as drug carriers to deliver entrapped drug moleculesinto or across the skin; act as penetration enhancers owing the penetration ofthe individual lipid components into the stratum corneumand subsequently the alteration of the intercellularlipid lamellae within this skin layer. (bouwstra 2005)

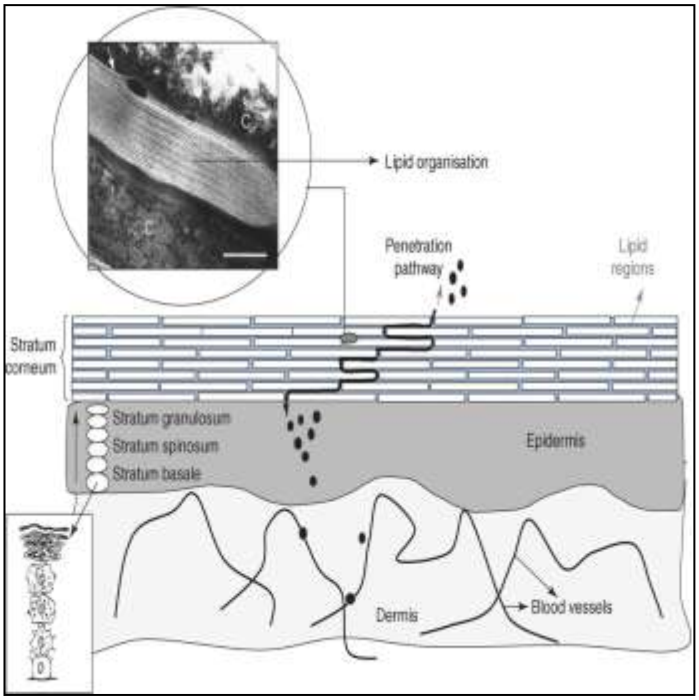

Figure 4: A schematic drawing of a skin cross-section

(c) serve as a depot for sustained release of dermally active compounds;

(d) serve as a rate-limiting membrane barrier for the modulation of systemic absorption, hence providing a controlled transdermal delivery system.

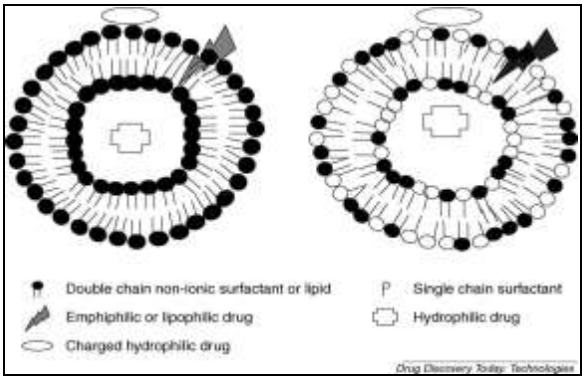
Double chain non-ionic surfactant or lipid
Single chain surfactant
Emphiphilic or lipophilic drug
Hydrophilic drug
Charged hydrophilic drug
Drug Discovery Today: Technologies

Figure 5: Rigid vesicles consist of double chain

non-ionic surfactants or lipids in the presence or absence of cholesterol (left image). Elastic vesicles consist of double chain surfactants or lipids and an edge activator. The edge activator is often a single chain surfactant (right image).

Despite extensive research and a great interest from thepharmaceutical and cosmetic industry, the use of vesicles isyet controversial. Two major questions are subjects for discussion:

(1) What is the effectiveness of vesicles?

(a) Do they enhance drug deposition in the skin (dermal delivery)?

(b) Do they enhance drug transport across the skin? (Transdermal delivery)?

(c) Or do they enhance both dermal and transdermal Delivery?

(2) What is the interaction between vesicles and the skin?

(a) Do they act as penetration enhancers?

(b) Or do they act as drug carrier systems?

The answers to the two questions above are required to

obtain a sound understanding of the mechanism of the actionof vesicles and to select themost appropriate drug compoundsto be delivered by vesicles. If vesicles act as carrier systems, theymight be able to transport large molecular weight drugs, suchas proteins intothe skin or even int o the systemic circulation. Ifthey act as penetration enhancers, however, the mainmode ofaction is a perturbation of the lipid organization in the stratumcorneum, thereby increasing the transport rate across the skin.The latter is only efficient for lowmolecular weight drugs.Oneof themost important characteristics of drug carrier systems isthat drug and carrier should permeate along the same routeacross the skin. In addition the vesicle material profileand the active compound profile in stratum corneum shouldbe very similar. (bouwstra 2005)

The effectiveness of conventional vesicles as skin drug delivery vehicles:

Early studies in the 1980sThefirst papers to report onthe effectiveness of vesicles for skindelivery were published in the early 1980s. The reported data, however, were conflicting. Mezei and Gulasekharam reportedthat liposomal encapsulation of triamcinolone acetonideincreased drug disposition in the epidermis and dermis. Several other groups suggested slower skin transport of highly polar compounds in vesicle formulations than inbuffer solutions. The transport rate of lipophilic compoundswas reported to be very similar to that of free drug solutions.Most groups concluded that liposomes did not act as transportsystem Conflicting results continued to be published concerningthe effectiveness of vesicles, enhancing the controversy of vesicles as (Trans) dermal delivery vehicles. Several transportstudies have reported that vesicles only enhanced the drugdisposal in the skin, suggesting that vesicles are only usefulfor topical dermal delivery. Others, however, havesuggested that application of drugs in vesicles could lead totherapeutic drug concentrations in the systemic circulationand are suitable candidates for transdermal deliveryThese inconsistent results can,at least in part, be explained bythe fact that vesicles with different compositions and physico-chemical characteristics were used in different studies. Solet us explain the physico-chemical characteristics of thevesicles in relation to their enhancing properties.The effect of conventional vesicles on drug transportSeveral studies have demonstrated that the vesicle compositionand hence its physico-chemical properties can have asignificant effect on drug permeation Several studies have demonstrated consistently that the phases ofthe amphiphiles formed in the membranes of the vesicles(a liquid or a gel phase) is an important feature, which plays avital role in its effectiveness as a(trans)dermal delivery vehicle.Permeation studies in vitro have revealed that LIQUID-STATEVESICLES are more effective than GEL-STATE VESICLES in enhancingdrug transport These results have recently been confirmed in vivo.Other physico-chemical properties, such as size and lamellarity,might also influence the effectiveness of vesicles as adelivery vehicle, although probably to a lesser extent than thethermodynamic state The mode of action of conventional vesiclesSeveral mechanisms mediating the vesicle–skin interactionshave been described in the literature. It has been suggestedthat vesicle–skin interactions can occur either at the skinsurface or in the deeper layers of the stratum corneum. Hoflandet al. and Abraham et al. have demonstrated adsorptionand fusion of vesicles onto the skin surface, resulting in theformation of lamellae and rough structures on top of theoutermost corneocytes .Changes in the deeper layersof the stratum corneumwere observed only after treatment ofthe skin with liquid-state liposomes and non-ionic surfactantvesicles. No ultrastructural changes in the skin were foundwhen gel-state non-ionic surfactant vesicles were applied. The authors explained their results by a molecularlydispersed penetration of lipid or surfactant intotheinterc ellularmatrix.Studies withthermal analysis thatenable to detectlipid phase transitions confirmed this mechanism. This suggests that components of liquid state vesicles can enter thedeeper layers of the stratum corneum where they can modifytheintercellularlipidlamellae, whereas the components of gelstatevesicles remain on the skin surface. The superior mode ofaction of liquid-state vesicles for skin interactions is the mostprobable explanationfor the fact that they aremore effective inenhancing drug transport into and across the skin. This is inaccords with a study that found a correlation between the skinpenetration and the fluidity of the vesicle bilayers determinedby electron spin resonance From the studies above there is no doubt that vesicularcomponents can penetrate into the stratum corneum. However, it is still debated whether vesicles can enter the stratumcorneum as intact entities. In fact only one group claimedthat liposomes enter the stratum corneum intactly .Remarkably, it was noticed that these liposomes are 'veryflexible lipid vesicles'. As explained below, an increaseddeformability of the bilayers will have consequences for theirinteraction with skin.In general, one can conclude that rigid liquid- and gel-statevesicles do not enter the stratum corneum as intact entities.However, the question remains whether highly deformablevesicles are able to enter the stratum corneum when appliedin an optimal manner. (melinda 2002)

Methods for Preparation of Tdds:

Asymmetric TPX membrane method:

A prototype patch can be fabricated for this a heat sealable polyester film (type 1009, 3m) with a concave of 1cm diameter will be used as the backing membrane. Drug sample is dispensed into the concave membrane, covered by a TPX {poly (4-methyl-1-pentene)} asymmetric membrane, and sealed by an adhesive. (Robert 2004)

Asymmetric TPX membrane preparation:

These are fabricated by using the dry/wet inversion process. TPX is dissolved in a mixture of solvent (cyclohexane) and nonsolvent additives at 60°c to form a polymer solution. The polymer solution is kept at 40°C for 24 hrs and cast on a glass plate to a pre-determined thickness with a gardener knife. After that the casting film is evaporated at 50°C for 30 sec, then the glass plate is to be immersed immediately in coagulation bath [maintained the temperature at 25°C]. After 10 minutes of immersion, the membrane can be removed, air dry in a circulation oven at 50°C for 12 hrs. (D.Patel 2011)

Traditional transdermal patches:

While infusion pumps are reliable in achieving a preferred therapeutic delivery profile,the use of such a system (e.g. an insulin pump) is somewhat cumbersome, requirestraining, is costly, and requires a hypodermic needle-based infusion set. Transdermalpatches, where the drug diffuses through the skin, offer a much more convenient wayto administer a drug while still having the benefits of continuous drug release.Transdermal patches were introduced in the late 1970s, starting with a threedaypatch to treat motion sickness. Since then, the market for drug administrationthrough patches has been steadily increasing and by 2004 the annual U.S. marketvalue was more than 3 billion with several kinds of drug formulations available In 2001, 51 of 129 drug delivery products under clinical evaluation in the U.S. weretransdermal or dermal systems. Still, only about eleven drugs are presentlyavailable with transdermal patches.. The fundamental reason why sofew drugs are used is that the barrier property of the skin limits the use of patchesto therapeutics where the molecule size is small enough to diffuse through the skinat therapeutic rates. The drugs presently used in patches have molecular massesranging from 162 u (nicotine) to 357 u (oxybutynin), giving a practical dose rate of4–20 mg/day depending on the patch size. As a comparison, the mass of the insulinmolecule is 5808 u and modern DNA-based vaccines, built of vectors with thousandsof base-pairs, may have molecular weights in the order of hundreds of kilounits (kDa).Transdermal patches are generally divided into two categories based on their phys-ical structure: reservoir-based and matrix-based. Reservoir-based patches hold thethe drug in a solution (usually a liquid or a gel) in a separate compartment. Thedrug is released through a rate-controlling permeable membrane placed as an interfacebetween the reservoir and the skin. Matrix-based patches have a more simple design in which thedrug is incorporated with the adhesive layer. There is no membrane that controlsthe release rate of the drug. Instead, the permeability of the skin governs the ratecontrol. Matrix-based patches are easier to fabricate and thus the production cost islower than for reservoir-based patches. On the other hand, reservoir-based patchesoffer better control of the drug release but may raise safety concerns since a possiblerupture of the membrane could result in a sudden release of the drug. (bouwstra 2005)

Alternative techniques for transdermal delivery

1. Jet injectors

Jet injectors are hand-held devices that deliver a high-pressure liquid stream througha small nozzle orifice. The impact of the stream is high enough to penetrate the skintissue and by controlling the magnitude, the liquid can be delivered to specific tissuedepths, e.g.intradermal, subcutaneous or intramuscular. The major advantage of jetinjectors is the extremely efficient way of drug administration where drug doses can befired off sequentially, allowing up to 1000 subjects to be medicated per hour [15]. Jetinjectors have been used in military and mass vaccination campaigns since the 1950sbut their use was discontinued after an outbreak of hepatitis B in 1985 was linkedto the use of jet injectors [16]. With the emergence of HIV, the use of jet injectors became too much of a risk and ceased definitely in 1997 as the U.S. Department ofDefense announced that they would stop their use.During recent years, with renewed interest due to bioterror threats, a new generationof safer jet injectors have been developed which use single-dose cartridgesand, for example disposable caps to eliminate the risk of cross-contamination betweeninjections. Several systems are commercially available, e.g. Bioject, Injex,Intraject, and J-Tip. A similar system called PMED also exists where the jetinjectedsubstance has the form of a dry powder (PowderMed Ltd, a Pfizer Inc.subsidiary) and where the delivery specifically targets immuno-competent cells in theskin layer. Due to this efficient location of vaccine delivery (cf. sec 2.6), immune responsehas been achieved with 20–2500-fold lower doses as compared to conventionalintramuscular delivery (using a needle and syringe) In addition, a big advantageof powder-based formulations of vaccines is that the so-called “cold-chain” could beeliminated The “cold-chain”, meaning an uninterrupted storage of drugs between2–8 ◦C throughout the whole supply chain, imposes major complications andadditional costs especially for vaccinations in more remote areas.While jet injectors are especially suited for mass vaccinations, injectors for insulindelivery have also been evaluated and commercialized (Medi-Jector) Likestandard needle-based injections, jet injectors deliver a bolus shot and the injectioncan cause pain. Some injectors however, are specifically designed to cause small orno pain. A system for continuous jet delivery using a piezoelectric actuator has also been proposed. (fung 1989)

2. Iontophoresis

Iontophoresis refers to the delivery of drugs across the skin by means of an electricfield. By having two electrodes placed on the skin, drugs at the electrodes will startto migrate through the skin once a voltage is supplied to the electrodes. Once in theskin, the drug will be absorbed by the capillaries and systemically distributed. Thecurrent density is usually below 0.5 mA/cm^2 in order not to cause the patient anydiscomfort. Three main physical mechanisms are involved in iontophoresis: chargedspecies are driven from the electrodes as a result of the electric field, i.e. electrophoresis; the flow of current increases thepermeability of the skin; and, the establishedpotential difference between the electrodes give rise to an electroosmotic flow .Since electroosmosis occurs, uncharged species can be delivered as well.The efficiency of iontophoretic mass transport is closely related to the propertiesof the drug. Polarity, valency and mobility of the drug molecules are essential, withthe mobility being tightly linked to the size of the molecule. As a consequence, iontophoresis is considered to be a feasible procedure for molecules of less than 7000 u.The flux of small molecules below 1000 u is typically 20–50 mg/day, whereas moleculesabove 5000 u generally leads to less than 1 mg/day. However, effective transportat rates of 6 mg/day · cm^{-2} has been reported even for 12000 u-sized proteins.There is several iontophoretic delivery systems commercially available. Most ofthem are electrode-based system with benchtop control units that require the physicianto add a drug to a reservoir on the electrode. In 2004, Vyteris Inc. received an NDA (New DrugApplication) approval from theFDA for a lidocaine-prefilled iontophoretic transdermal system used for local analgesia (pain relieve) in clinics. This system consists of a disposable drug-filled patchwhich is electrically connected to a reusable, portable and battery-operated, controller.In 2006, Alza corp. received an NDA on a fully integrated iontophoreticpatch prefilled with fentanyl (pain relief). Based on Alza's E-Trans platform, the disposable patch system, called Ionsys, incorporates drug, battery, LEDs (LightEmitting Diodes) and an ASIC (Application Specific Integrated Circuit), The system is activated and operated by the patient and each activation renders a40 g dose, delivered within 10 min. The ASIC ensures proper usage by the patientand compensates for physiological variations by controlling the iontophoreticcurrent. Another specific application field for iontophoresis is diabetes care. Although notused for drug delivery, non-invasive glucose monitoring by means of iontophoresishas been demonstrated and commercialized. The GlucoWatch, a wrist-worndevice, uses iontophoretic current to sample glucose from the interstitial fluid in theskin. By incorporating a replaceable enzymatic (glucose oxidase) glucose sensor in thedevice, the blood glucose of the patient can be estimated and continuously recorded.Delivery of insulin (5808 u) using iontophoresis has also been investigated, cf.and references therein. However, basal rates of insulin needed by diabetics is 0.5–1 mg/day and even this low basal drug input exceeds the theoretical flux predictedfor iontophoretically delivered monomeric insulin on a 10 cm^2 area (H.williamann 1992)

3. Sonophoresis

Another method to move drugs across the skin barrier is sonophoresis where skin ismade permeable under influence of ultrasonic waves. The technique has been usedfrequently for over half a century, e.g. with hydrocortisone in combination with physicaltherapy of joint-related complications (*arthritis*). Traditionally, frequenciesabove 1 MHz have been used in order to reach and simultaneously stimulate tissues (joints and muscles) below the skin. However, during the past decade a considerableamount of research has been directed towards low-frequency sonophoresis (<100 kHz) shown to interact with the superficial skin tissue and increasing transdermal transportby orders of magnitude. Thermal, chemical and mechanical alterations in theskin are considered to be the main transport enhancing mechanism in sonophoresis.For low-frequency sonophoresis, the formation and collapse of bubbles within the cells (cavitation) causes disruption of the skin tissue and are believed to be the predominanteffect by which the method works. Administrations of many different drugs, e.g.insulin, low-molecular weight heparin, and vaccine, have been demonstrated feasiblewith low-frequency sonophoresis. Typical acoustic intensities range from0.25–1 W/cm^2.In 2004, Sontra Medical Corp. (a spin off from R. Langer's lab at MIT) receivedFDA approval for the first sonophoretic transdermal delivery system. Thesystem, SonoPrep, is aimed for lidocaine administration (pain relief) and consistsof a portable base unit connected to an ultrasonic horn that is pressed onto the area ofskin to be treated. The company is also developing an non-invasive glucose monitoringsystem based on sonophoretic disruption of the skinreceived an NDA (New Drug Application) approval from theFDA for a lidocaine-prefilled iontophoretic transdermal system used for local analgesia(pain relieve) in clinics. This system consists of a disposable drug-filled patchwhich is electrically connected to a reusable, portable and battery-operated, controller.In 2006, Alza corp. received an NDA on a fully integrated iontophoreticpatch prefilled with fentanyl (pain relief). Based on Alza's E-Trans platform,the disposable patch system, called Ionsys, incorporates drug, battery, LEDs (LightEmitting Diodes) and an ASIC (Application Specific Integrated Circuit), The system is activated and operated by the patient and each activation renders a 40 g dose, delivered within 10 min. The ASIC ensures proper usage by the patientand compensates for physiological variations by controlling the iontophoreticcurrent another specific application field for iontophoresis is diabetes care. Although notused for drug delivery, non-invasive glucose monitoring by means of iontophoresishas been demonstrated and commarcial The GlucoWatch, a wrist-worndevice, uses iontophoretic current to sample glucose from the interstitial fluid in theskin. By incorporating a replaceable enzymatic (glucose oxidase) glucose sensor in thedevice, the blood glucose of the patient can be estimated and continuously recorded.Delivery of insulin (5808 u) using iontophoresis has also been investigated, cf. and references therein. However, basal rates of insulin needed by diabetics is 0.5–1 mg/day and even this low basal drug input exceeds the theoretical flux predictedfor iontophoretically delivered monomeric insulin on a 10 cm^2 area.

Evaluation parameter:-

1. Interaction studies:

Excipients are integral components of almost all pharmaceutical dosage forms. The stability of a formulation amongst other factors depends on the compatibility of the drug with the excipients. The drug and the excipients must be compatible with one another to produce a product that is stable, thus it is mandatory to detect any possible physical or chemical interaction as it can affect the bioavailability and stability of the drug. If the excipients are new and have not been used in formulations containing the active substance, the compatibility studies play an important role in formulation development. Interaction studies are commonly carried out in Thermal analysis, FT-IR, UV and chromatographic techniques by comparing their physicochemical characters such as assay, melting endotherms, characteristic wave numbers, absorption maxima etc. Thermal analysis, FT-IR, UV and chromatographic techniques by comparing their physicochemical characters such as assay, melting endotherms, characteristic wave numbers, absorption maxima etc. (Heather 2005)

2. Thickness of the patch:

The thickness of the drug loaded patch is measured in different points by using a digital micrometer and determines the average thickness and standard deviation for the same to ensure the thickness of the prepared patch. (Heather 2005)

3. Weight uniformity:

The prepared patches are to be dried at 60°c for 4hrs before testing. A specified area of patch is to be cut in different parts of the patch and weigh in digital balance. The average weight and standard deviation values are to be calculated from the individual weights. (Heather 2005)

4. Folding endurance:

A strip of specific are is to be cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance. (Heather 2005)

5. Percentage Moisture content:

The prepared films are to be weighed individually and to be kept in a desiccators containing fused calcium chloride at room temperature for 24 hrs. After 24 hrs the films are to be reweighed and determine the percentage moisture content from the below mentioned formula.

Percentage moisture content = [Initial weight- Final weight/ Final weight] ×100 (Heather 2005)

6. Percentage Moisture uptake:

The weighed films are to be kept in a desiccators at room temperature for 24 hrs containing saturated solution of potassium chloride in order to maintain 84% RH. After 24 hrs the films are to be reweighed and determine the percentage moisture uptake from the below mentioned formula. (Heather 2005)

Percentage moisture uptake = [Final weight- Initial weight/ initial weight] ×100

7. Water vapour permeability (WVP) evaluation:

Water vapour permeability can be determined with foam dressing method the air forced oven is replaced

by a natural air circulation oven. The WVP can be determined by the following formula
WVP=W/A
Where, **WVP** is expressed in gm/m2 per 24hrs,
W is the amount of vapour permeated through the patch expressed in gm/24hrs and
A is the surface area of the exposure samples expressed in m2 (Heather 2005).

8. Drug content:

A specified area of patch is to be dissolved in a suitable solvent in specific volume. Then the solution is to be filtered through a filter medium and analyze the drug contain with the suitable method (UV or HPLC technique). Each value represents average of three different samples. (Heather 2005)

9. Uniformity of dosage unit test:

An accurately weighed portion of the patch is to be cut into small pieces and transferred to a specific volume volumetric flask, dissolved in a suitable solvent and sonicate for complete extraction of drug from the patch and made up to the mark with same. The resulting solution was allowed to settle for about an hour, and the supernatant was suitably diluted to give the desired concentration with suitable solvent. The solution was alysed by suitable analytical technique (UV or HPLC) and the drug content per piece will be calculated. (Heather 2005)

10. Polariscope examination:

This test is to be performed to examine the drug crystals from patch by polariscope. A specific surface area of the piece is to be kept on the object slide and observe for the drugs crystals to distinguish whether the drug is present as crystalline form or amorphous form in the patch. (Heather 2005)

11. Peel Adhesion test:

In this test, the force required to remove an adhesive coating form a test substrate is referred to as peel adhesion. Molecular weight of adhesive polymer, the type and amount of additives are the variables that determined the peel adhesion properties. A single tape is applied to a stainless steel plate or a backing membrane of choice and then tape is pulled from the substrate at a 180º angle, and the force required for tape removed is measured. (Heather 2005)

12. Thumb tack test:

It is a qualitative test applied for tack property determination of adhesive. The thumb is simply pressed on the adhesive and the relative tack property is detected. (Heather 2005)

13. Flatness test:

Three longitudinal strips are to be cut from each film at different portion like one from the center, other one from the left side, and another one from the right side. The length of each strip was measured and the variation in length because of non-uniformity in flatness was measured by determining percent constriction, with 0% constriction equivalent to 100% flatness. (Heather 2005)

14. Percentage Elongation break test:

The percentage elongation break is to be determined by noting the length just before the break point, the percentage elongation can be determined from the below mentioned formula.
Elongation percentage = L1-L2 ×100 L2
Where, **L1**is the final length of each strip and
L2 is the initial length of each strip. (Heather 2005)

15. Rolling ball tack test:

This test measures the softness of a polymer that relates to talk. In this test, stainless steel ball of 7/16 inches in diameter is released on an inclined track so that it rolls down and comes into contact with horizontal, upward facing adhesive. The distance the ball travels along the adhesive provides the measurement of tack, which is expressed in inch. (Heather 2005)

16. Quick Stick (peel-tack) test:

In this test, the tape is pulled away from the substrate at 90ºC at a speed of 12 inches/min. The peel force required to break the bond between adhesive and substrate is measured and recorded as tack value, which is expressed in ounces or grams per inch width. (Heather 2005)

17. Probe Tack test:

In this test, the tip of a clean probe with a defined surface roughness is brought into contact with adhesive, and when a bond is formed between probe and adhesive. The subsequent removal of the probe mechanically breaks it. The force required to pull the probe away from the adhesive at fixed rate is recorded as tack and it is expressed in grams. (Heather 2005)

18. *In vitro* drug release studies:

The paddle over disc method (USP apparatus V) can be employed for assessment of the release of the drug from the prepared patches. Dry films of known thickness is to be cut into definite shape, weighed, and fixed over a glass plate with an adhesive. The glass plate was then placed in a 500-mL of the dissolution medium or phosphate buffer (pH 7.4), and the apparatus was equilibrated to 32± 0.5°C. The paddle was then set at a distance of 2.5 cm from the glass plate and operated at a speed of 50 rpm. Samples (5- mL aliquots) can be withdrawn at appropriate time intervals up to 24 h and analyzed by UV spectrophotometer or HPLC. The experiment is to be performed in triplicate and the mean value can be calculated. (Heather 2005)

19. Skin Irritation study:

Skin irritation and sensitization testing can be performed on healthy rabbits (average weight 1.2 to 1.5 kg). The dorsal surface (50cm2) of the rabbit is to be cleaned and remove the hair from the clean dorsal surface by shaving and clean the surface by using rectified spirit and the representative formulations can be applied over the skin. The patch is to be removed after 24 hr and the skin is to be observed and classified into 5 grades on the basis of the severity of skin injury. (Heather 2005)

20. Stability studies:

Stability studies are to be conducted according to the ICH guidelines by storing the TDDS samples at 40±0.5°c and 75±5% RH for 6 months. The samples were withdrawn at 0, 30, 60, 90 and 180 days and analyze suitably for the drug content. (Heather 2005)

Transdermal Drug Delivery System Market:

Table 1gives a list of companies in transdermal drug delivery systems with their current products and technologies in the market. 3M Pharmaceuticals is a leader in pioneering the technological components in transdermal drug delivery system and components made by 3M are used for manufacturing the complete spectrum of drugs delivered transdermally. **Figure 6** shows a graph showing the range of transdermal drug delivery system currently sold in the market. On the X-axis are the drugs that are administered transdermally, while the Yaxisin the graph shows percentage of the total transdermal products that are being sold in the market.

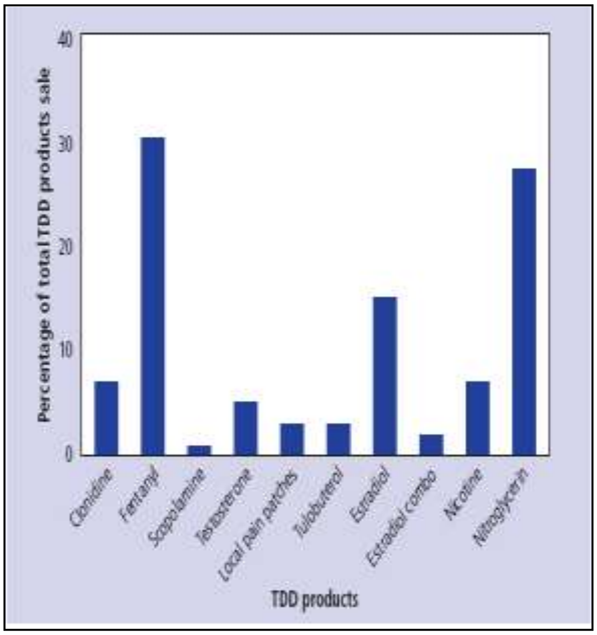

Figure 6: Graph of Transdermal Drug Delivery Products Vs. Percentage Total Sold

Transdermal Drug Delivery Products

The range of the current drugs that are administered by using Transdermal drug delivery technique is still limited due to the skin posing as a barrier against the drug diffusion or permeation. This barrier of stratum corneum is overcome by use of active transdermal drug delivery techniques that are relatively new to the market but are considered to have great potential towards increasing the range and variety of drugs that could be administered transdermally. The annual market for transdermal Drug Delivery systems is more than $3 billion.

Company Name Transdermal Products/Technology in Market:

Table 1: Companies and their Transdermal Drug Delivery Technologies & Products

Company Name	**Transdermal Products/Technology in Market**
3M Pharmaceuticals	MinitranTM, Pioneer in the field of Manufacturing Transdermal Components
Acrux Ltd.	ACROSSR, MDTSR, Patchless PatchR
Adhesives Research, Inc.	ETATM, HRT Adhesives, PIB Adhesives, MTT Adhesives
Adherex Technologies, Inc	ExherinTM
Altea Therapeutics Corp.	PassPortR Patch
Alza Corp.	D-TransR System, E-TransR System, MacrofluxR System
Antares Pharma, Inc.	ATDTM Gel Technology
Biochemics, Inc.	PENtoCORER
Boehringer Ingelheim Corp.	CATAPRES-TTSR
Dermisonics, Inc.	U-Strip
Elan Transdermal Technologies, Inc.	Buspirone Patch
Inovio Biomedical Corp	MedPulser Electroporation Therapy System
ImaRx Therapeutics, Inc	SonoDermTM Technology
Iomed, Inc.	Iontophoreris Electrodes: TransQR Flex, IOGELR, TransQR E, OptimaAR ; Numby StuffR, IONTOCAINER, PhoresorR
Noven Pharmaceuticals, Inc.	VivelleR, Vivelle-DotTM, CombiPatchTM, EstalisR, MethyPatchR
L.A.M. Pharmaceuticals LLC	IPM Wound GelTM, Polymer MatrixTM Technology
Macrochem Corp.	MacroDermTM, SEPAR
Norwood Abbey Ltd.	Laser Assisted Drug Delivery(LAD), Micro-needle Drug Delivery
Sontra Medical Corp.	SonoPrepR
Vyteris, Inc.	LidoSiteTM Topical System

FDA Regulation and Transdermal Drug Delivery System:

The first FDA approved Transdermal Drug Patch was in the year 1979. Since then, the transdermal drug delivery systems have come a long way. It gives a chronology of the events in the field of transdermal technology along with the approvals that were obtained at every stage of this chronology.The FDA regulation process for Transdermal drug delivery system is very stringent.Transdermal Drug Delivery system is a combinational device as defined in 21 CFR § 3.2(e) byFood and Drug Administration. Transdermal drug delivery system have to undergo premarket approval (PMA) and hence requires substantial evidence including biomechanical testing,animal testing, clinical trials studies before the transdermal patch can get approval for use in the market. The most recent approval in the field of transdermal drug delivery system was the approval of Nuepro patch for treatment of Parkinson's disease.

In the case of Passive transdermal drug delivery system, the factor that requires considerationis ensuring that the drug in the drug-reservoir or the drug-in-adhesive is present and beingdelivered in a stable as well as controlled form.) It is also important to understand the reactivity of the drug on the skin and ensure that the material used for manufacturing the transdermal patchdo not have an adverse reaction on the skin, for instance itching, inflammation, etc. The patchalso needs to be kept on from several hours to, in some cases, several days (e.g., contraceptive patch) and hence the properties of the patch like the type of polymers, adhesives used in themaking also need special consideration. The material used for making the patch is polymers.There are various types of polymeric materials that are utilized for the construction of transdermal drug delivery system. The following section in the paper describes the polymeric materials along with their properties that are used in the making of transdermal drug delivery system (Joshi 2008)

Future Considerations:

In terms of technical advances in transdermal drug delivery systems, with the advent of penetration enhancers, the future for transdermal drug delivery system looks bright. The use of "X-ray lithography (DXRL) and LIGA (German acronym: LIthographie-Galvanoformung- Abformung) technology" that uses a micro-gear pump and an array of microneedles is one ofthe latest developed penetration enhancer technique. This transdermal patch is still built of polymeric material (polymethylmethacrylate) PMMA. X-ray Lithorgraphy and LIGA based Transdermal Drug Delivery System Though the system flow rate and flow control was controlled with this type of deliverysystem, the system still needs to work on the type of skin penetration since there was a lot of skin rupture observed in the testing of this system. The other issue observed was that of system being mechanically unstable and hence this technique is not in the market yet. But with some modification and research, X-ray lithography and LIGA based transdermal drug delivery system will surely be of importance. With all the new techniques like use of ultrasound, heat, electroporation, etc. as aids to penetration enhancer, the basic issue with the skin posing as barrier to drug permeation will be an issue of the past.In terms of the material advances, there are advances undergoing currently in terms of use ofcomposite cellulose membrane for selective controlled drug delivery. One such study was done by Suedee et.al. (2008), in which they used molecularity imprinted polymer (MIP) that is a thinlayercellulose membrane with selectivity for S-propranol employed as enantioselectivecontrolledrelease system. The study was reported successful after in vivo studies, and concluded that the use of MIP in rats was similar to transdermal gel patch.The advantages of transdermal drug delivery system include "reduced frequency of administration thereby increasing patient compliance, avoidance of gastric metabolism, and hepatic first-pass metabolism and the achievement of steady state plasma concentration levelsand avoidance of high peak levels, due to continuous drug delivery, which may reduce systemicside effects". But there are still inherent side effects related to the use of transdermal systems and they are adverse reaction on skin. Study done by Bennett (1998) showed that there are issues with skin reaction ranging from inflammation, etching, dryness, redness, to rash and in certain cases burns on the patch applied areas. There is still a lot of improvement that needs to be done in this area to avoid skin reaction on the applied patch area and there is constant search going on for better and more biocompatible materials for effective and safe drug delivery (Joshi 2008).

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PARTICULATE DRUG DELIVERY SYSTEM – LIPOSOMES

Satam Madhavi, Hastak Vishakha

Introduction:

A liposome is a spherical vesicle with a membrane composed of a phospholipid bilayer used to deliver drug or genetic material into a cell.
(Anwekar H JULY 2011)

Advantages of Liposome:

1. Liposome increases efficacy and therapeutic index of drug .
2. Liposome increases dtug stability via encapsulation.
3. Liposomes are biocompatible, completely biodegradable, non-toxic, flexible and nonimmunogenic for systemic and non-systemic administrations.
4. Liposome helps in reduction in toxicity of the encapsulated agent.
5. Liposomes help to reduce exposure of sensitive issues to toxic drugs.
6. Site avoidance effect.
7. Flexibility to couple with site-specific ligands to achieve active targeting.

(Anwekar H JULY 2011)

Disadvantages of Liposome:

1. Production cost is high.
2. Leakage and fusion of encapsulated drug molecules.
3. Sometimes phospholipid undergoes oxidation and hydrolysis like reaction.
4. Short half-life.
5. Low solubility
6. Fewer stables.

(Anwekar H JULY 2011)

Structural Components of Liposome:

There are number of the structural and nonstructural components of liposomes, major structural components of liposomes are:

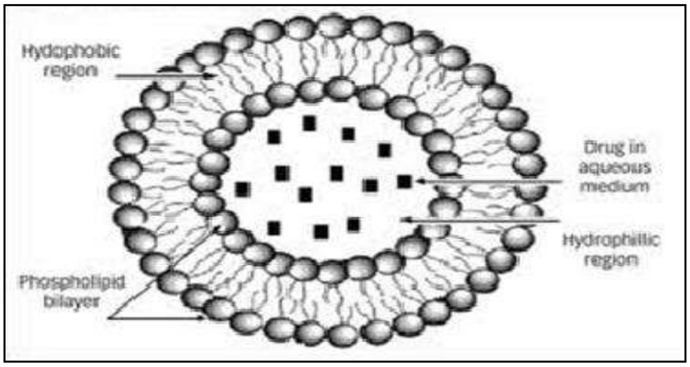


Figure 1: Structure of liposome: (Prasanath.V.V 2012)

a. Phospholipids:

Phospholipids are the major structural component of biological membranes, where two type of phospholipids exit- PHOSPHODIGLYCERIDES AND SPHINGOLIPIDS. The most common molecule. Molecule of phosphatidylcholinc are not soluble in water and in aqueous media they align themselves closely in plannar bilayer sheets in order to minimize the unfavorable action between the bulk aqueous phase and long hydrocarbon fatty chain. The Glycerol containing phospholipids are most common used component of liposome formulation and represent greater than 50% of weight of lipid in biological membranes. These are derived from Phosphatidic acid.

Examples of phospholipids are:

1. Phosphatidyl choline (Lecithin) – PC
2. Phosphatidyl ethanolamine (cephalin) – PE
3. Phosphatidyl serine (PS)
4. Phosphatidyl inositol (PI)
5. Phosphatidyl Glycerol (PG)

b. Cholesterol:

Cholesterol dose not by itself form bilayer structure, but can be incorporated into phospholipid membranes in very high concentration up to 1:1 or even 2:1 molar ration of cholesterol to phosphatidylcholine. Cholesterol inserts into the membrane with its hydroxyl group oriented towards the aqueous surface and aliphatic chain aligned parallel to the acyl chains in the center of the bilayer. The high solubility of

cholesterol in phospholipid liposome has been attributed to both hydrophobic and specific head group interaction, but there is no unequivocal evidence for the arrangement of cholesterol in the bilayer.
(Anwekar H JULY 2011), (Katyayani M.S 2011), (Fendler (1977))

Mechanism of Liposome Formation:

Phospholipids are amphipathic having affinity for both aqueous and polar moieties molecules as they have a hydrophobic tail and a hydrophilic or polar head. The hydrophobic tail is composed of two fatty acid chain containing 10-24 carbon atom and 0-6 double bonds in each chain. The macroscopic structures most often formed include lamellar, hexagonal or cubic phases dispersed as colloidal nanoconstructs (artificial membranes) referred to as liposomes, hexasomes or cubosomes. The most common natural polar phospholipids are phosphatidylcholinc. These are amphipathic molecules in which a glycerol bridge links to a pair of hydrophobic acyl hydrocarbon chains with a hydrophilic polar head group, phosphocholine. The amphipathic nature of phospholipids and their analogues render them the ability to form closed concentric bilayers in presence of water. Liposomes are formed when thin lipid films or lipid cakes are hydrated and stacks of lipid crystalline bilayers become fluid and swell. The hydrated lipid sheets detach during agitation and self close to form large, multilamellar vesicles prevent interaction of water with the hydrocarbon core of the bilayer at the edges.
(Anwekar H JULY 2011)

Classification of Liposomes:

Liposomes are classified on the basis of:
1. Structure.
2. Method of preparation.
3. Composition and application.
4. Conventional liposome.
5. Specialty liposome.

1. Classification Based on Structure: (Table 1)

Vesicle Type	**Abbreviation**	**Diameter Size**	**No of Lipid Bilayer**
Unilamellar vesicle	UV	All size range	One
Small Unilamellar vesicle	SUV	20-100 nm	One
Medium Unilamellar vesicle	MUV More than	100nm	One
Large Unilamellar vesicle	LUV	More than 100nm	One
Giant Unilamellar vesicle	GUV	More than 1 micro meter	One
Oligolamellar vesicle	OLV	0.1-1 micro meter	Approx. 5
Multilamellar vesicle	MLV	More than 0.5	5-25
Multi vesicular vesicle	MV	More than 1 micro meter	Multi compartmental structure

(Katyayani M.S 2011)**,** (Abdus Samad 2007), (Sharma.A 1997),(Fendler (1977)

2) Based on method of preparation: (Table2)

Preparation method	Vesicle type
Single or oligo lamellar vesicle made by reverse phase evaporation method	REV
Multi lamellar vesicle made by reverse phase evaporation method	MLV-REV
Stable pluri lamellar vesicle	SPLV
Frozen and thawed multi lamellar vesicle	FATMLV
Vesicle prepared by extrusion technique	VET
Dehydration- Rehydration method	DR V

(Katyayani M.S 2011)**,** (Abdus Samad 2007), (Sharma.A 1997),(Fendler (1977)

3) based on composition and application:(Table 3)

Type of liposome	abbreviation	Composition
Conventional liposome	CL	Neutral or negatively charged phospholipids and cholesterol
Fusogenic liposome	RSVE	Reconstituted Sendal virus envelope
Ph sensitive liposome	-	Phospholipids like Pes(1,2-diacyl-sn 226lycerol-3-phosphorylethanolamine, phosphatidylethanolamine) and DOPEs dioleoylphosphatidylethanolamine cholesterol/tetradecane with either CHEMS or OA
Cationic liposome	-	Positively charged cationic lipids with DOPE used to transport DNA
Long circulatory liposome	LCL	CL or LCL with attached monoclonal antibodies or recognition sequences
Immuno liposome	IL	Neutral high Tc, Chol and 5 – 10% of PEG – DSPE or GM1 Immuno liposomes

(Katyayani M.S 2011)**,** (Abdus Samad 2007), (Sharma.A 1997),(Fendler (1977)

Based Upon Conventional Liposome:

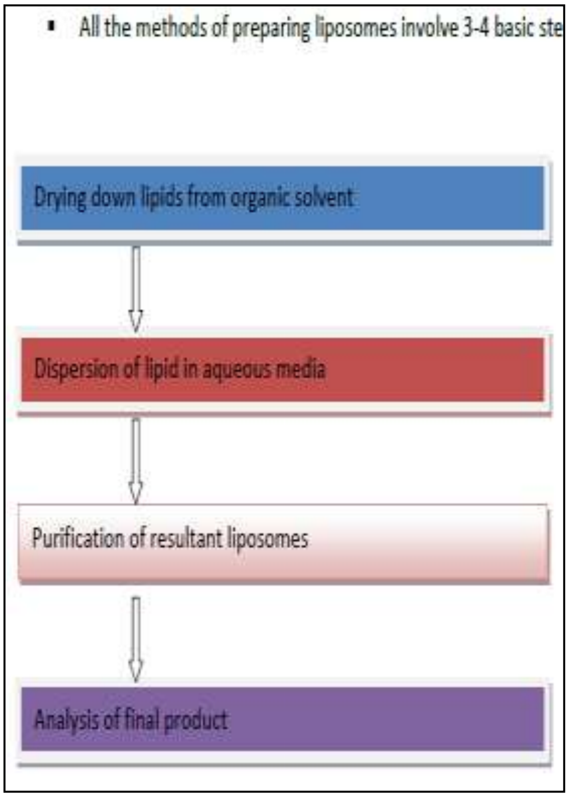
1- Stabilize natural lecithin (PC) mixtures
2- Synthetic identical, chain phospholipids
3- Glycolipids containing liposome

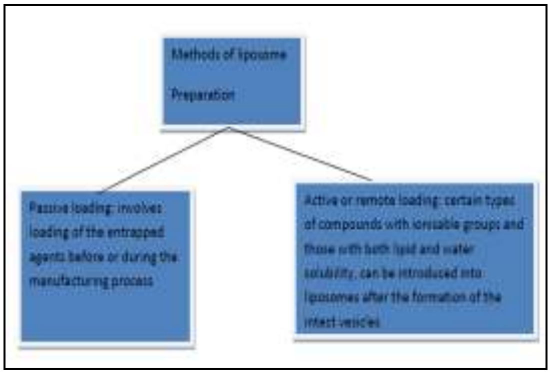
(Katyayani M.S 2011)**,** (Abdus Samad 2007), (Sharma.A 1997),(Fendler (1977)

Based Upon Speciality Liposome:

1- Bipolar fatty acid
2- Antibody directed liposome.
3- Methyl/ Methylene x- linked liposome.
4- Lipoprotein coated liposome.
5- Carbohydrate coated liposome.
6- Multiple encapsulated liposome.

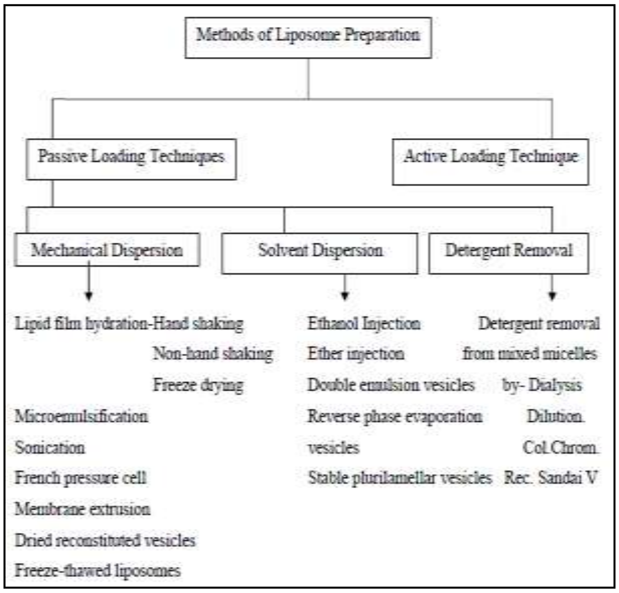
General Methods of Preparation:


All the methods of preparing liposomes involve 3-4 basic ste
Drying down lipids from organic solvent
Dispersion of lipid in aqueous media
Purification of resultant liposomes
Analysis of final product


Methods of liposome Preparation
Passive loading: involves loading of the entrapped agents before or during the manufacturing process
Active or remote loading: certain types of compounds with ionisable groups and those with both lipid and water solubility, can be introduced into liposomes after the formation of the intact vesicles

(Sharma Vijay K aug-oct 2010)

Method of Liposome Preparation and Drug Loading:



(Sharma Vijay K aug-oct 2010)

1. Passive loading techniques:

A] Mechanical dispersion method:

1)Lipid Hydration Method:

This is the most widely used method for the preparation of MLV. The method involves drying a solution of lipids so that a thin film is formed at the bottom of round bottom flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for some time. The hydration step is done at a temperature above the gel-liquid crystalline transition temperature Tc of the lipid or above the Tc of the highest melting component in the lipid mixture. The compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids depending upon their solubilities. MLV are simple to prepare by this method and a variety of substances can be encapsulated in these liposomes. MLVs with high encapsulation efficiency can be prepared by hydrating the lipids in the presence of an immiscible organic solvent (petroleum ether, diethyl ether). The contents are emulsified by vigorous vortexing and sonication. The organic solvent is removed by passing a stream of nitrogen gas over the mixture. MLVs are formed immediately in the aqueous phase after the removal of organic solvent. The main drawback of this method is the exposure of the materials to be encapsulated to organic solvent and to sonication. The drawbacks of the method are low internal volume, low encapsulation efficiency and the size distribution is heterogeneous.
(Prasanath.V.V 2012)

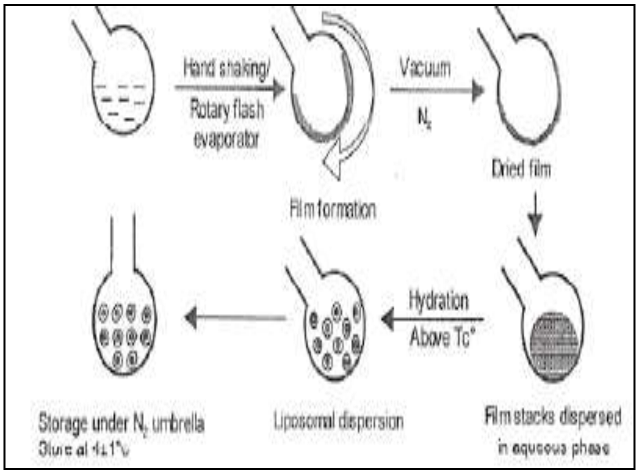


Figure 1:lipid hydration method: (Vyas S.P. n.d.)

2) Sonication method: This is the most common method used for the production of the small unilamellar liposomes (SUV). SUV'S are prepared by the sonication of the obtained MLV'S by conventional method. By this method SUV'S are obtained whose diameter range is 15-20 nm and this method helps to obtain homogenous SUV'S. Usually bath type sonicator and probe type sonicator are preferred and gases such as nitrogen and argon are used to maintain atmospheric pressure in the sonicator. The basic principle involved in the sonication are MLV'S suspensions are agitated under pulsed, high frequency sound waves (sonic energy), as mentioned above the sonication process produces homogenous SUV'S along with high tissue penetration. Mostly bath type sonicator is used for the preparation of the SLV'S. In this type a test tube is used in which MLV'S suspension is present and is placed in the bath sonicator for about 5-10 min for sonication. The sonicator is done at transitional temperature (i.e. the temperature at which the liquid melts). Bath sonication is the closed system in which it maintains the temperature and controls the temperature to minimize the thermo degradation of the lipid and entrapped substance.

(Prasanath.V.V 2012)**,** (Katyayani M.S 2011)

Advantages of bath sonicator over the probe tip sonicator:

1) Bath sonicator maintains the temperature, but the probe tip type sonicator produces over heat which causes degradation of the suspension

2)Break down of MLV'S to SLV'S process will be faster than probe sonicator.

3) As the probe sonicator contains the titanium particles and the aerosols containing carcinogenic agent. Radioactive agents will interact with the titanium particles and produces serious bio hazards.

2) French Pressure Cell Method:

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has several advantages over sonication method. The method is simple rapid, reproducible and involves gentle handling of unstable materials. The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of the method are that the temperature is difficult to achieve and the working volumes are relatively small (about 50 mL maximum).They deposited egg phosphatidylcholinc mixed with 1.5 %w/v of cetyl tetramethylammonium bromide (a detergent) in CHCI3/CH3OH on various supports for example silica gel powder, zeolite X, zeolite ZSM5. After the removal of organic phase, the system was resuspended by shaking or stirring in distilled water or 5 mM NaCl. There was some loss of phospholipid (about 10-20%) due to adsorption on the supports. The loss was 70% and 95% in the case of silica gel and zeolite ZSMS respectively. An homogenous population of vesicle with average diameter of 21.5 nm was obtained when zeolite X (particle size of 0.4 mm) was used as a support.

3) Freeze-Thaw Method

SUVs are rapidly frozen and followed by slow thawing. The brief sonication disperses aggregated materials to LUV. The formation of unilamellar vesicles is due to the fusion of SUV during the processes of freezing and or thawing . This type of fusion is strongly inhibited by increasing the ionic strength of the medium and by increasing the phospholipids concentration. The encapsulation efficiencies from 20 to 30% were obtained.

4)Reverse Phase Evaporation Method:

In this method organic solvents such as (diethyl ether / isopropyl ether or mixture of isopropyl ether and chloroform) are used which contains phospholipids in which the aqueous solution had to be injected. Sonication is done to form water-in-oil emulsion. The organic solvents are removed / evaporated under the reduced pressure to form viscous gel. The liposomes are formed when the residual solvent is completely removed from the mixture by prolonged and continuous evaporation process under reduced pressure. By this process LUV'S obtained are of the diameter 0.1-1μm. This process is used to encapsulate both small and large macromolecules including RNA and various enzymes. Denaturation of the proteins & breakage of the DNA / RNA strands due to the mechanical agitation.

(Prasanath.V.V 2012)**,** (Katyayani M.S 2011)

5) High-pressure extrusion method:

This is another method for converting MLV to SUV suspensions. By this method, suspensions of MLVs prepared by the convectional method are repeatedly passed through filters polycarbonate membranes with very small pore diameter (0.8–1.0μm) under high pressure up to 250psi. By choosing filters with appropriate pore sizes, liposomes of desirable diameters can be produced. The mechanism of action of the high pressure extrusion method appears to be much like peeling an onion. As the MLVs are forced through the small pores, successive layers are peeled off until only one remains. Besides reducing the liposome size, the extrusion method produces liposomes of homogeneous size distributions. A

variety of different lipids can be used to form stable liposomes by this method. Extrusion at low pressures <1Mpa is possible when lipid concentration is low, but the most commonly used pressures are about 5Mpa. A new technique uses 10.5Mpa for better results.
(Katyayani M.S 2011)

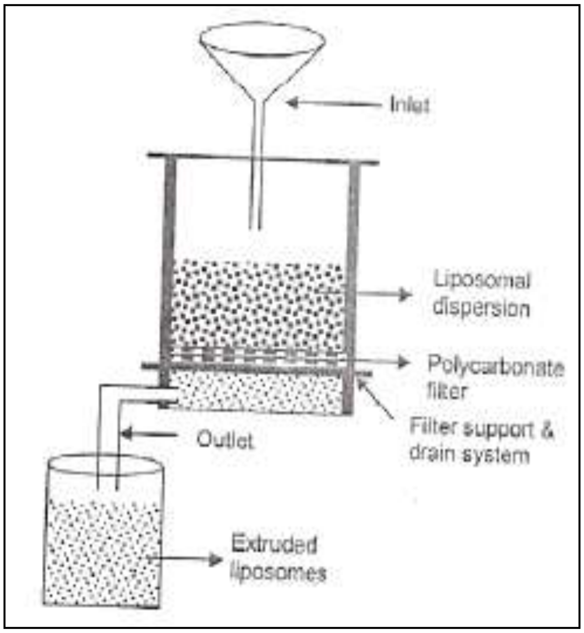


Figure 2: High-pressure extrusion method. (Vyas S.P.n.d.)

B] Solvent dispersion methods:

1) Ether Infusion Method:

A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that the population is heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature.
(Prasanath.V.V 2012)

2) Ethanol Injection Method:

A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all ethanol because it forms azeotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol.
(Prasanath.V.V 2012)

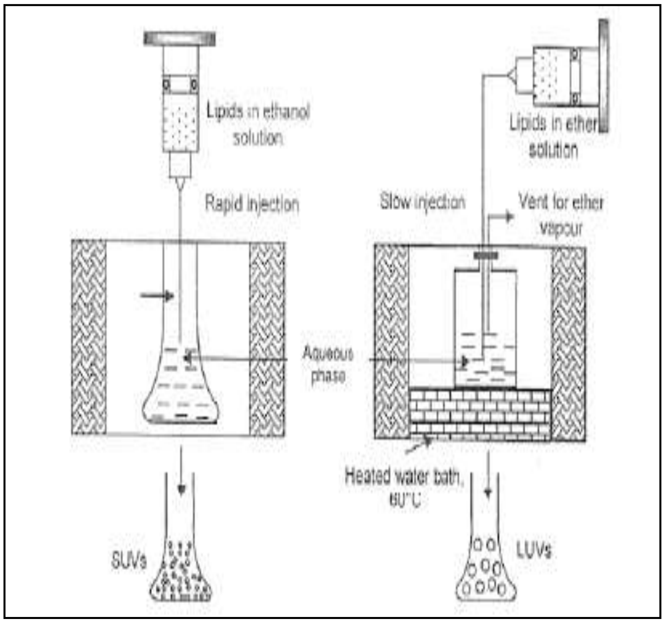


Figure 3: ethanol injection method and ether injection method. (Vyas S.P. n.d.)

C] Detergent Removal Methods:

This indicates that, the use of a detergent (surfactant) for the solubilisation of lipids. This is the main process in the preparation of LVS'S. The surfactant used is non-ionic (N-octyl-beta-D-gucopyranose), anionic surfactants (dodecyl sulphate) and cationic surfactants (hexa decyltrimethyl ammonium bromide). Mainly the detergent should possess higher miscellar concentration, to solubilise in the lipids and this is the basic principle behind the formulation. After the solubilisation of lipids in the detergent solution, the solution is removed by the dialysis /column chromatography10. By this process we can obtain the LUV'S of the diameter 0.08 - 0.2 µm. This process is mainly used for the encapsulation of the proteins of the biomedical importance.
(Prasanath.V.V 2012)

2. Active loading technique:

Industrial Production of Liposomes:

The several preparation methods described in the literature, only a few have potential for large scale manufacture of liposomes. The main issues faced to formulator and production supervisor are presence of organic solvent residues, physical and chemical stability, pyrogen control, sterility, size and size distribution and batch to batch reproducibility. Liposomes for parenteral use should be sterile and pyrogen free. For animal experiments, adequate sterility can be achieved by the passage of liposomes through up to approximately 400 nm pore size Millipore filters. For human use, precautions for sterility must be taken during the entire preparation process: that is, 1) the raw materials must be sterile and pyrogen free, 2) preparation in sterile system: working areas equipped with laminar flow and 3) use of sterile containers Some issues related to phospholipids need attention. The liposomes based on crude egg yolk phospholipids are not very stable. The cost of purified lipids is very high. Recently, liposomes have been prepared using synthetic and polymerizable lipids. The liposomes prepared from polymerizable phospholipids are exposed to UV light. The polymerization process takes place in the bilayer(s). Such liposome preparations usually have better storage stability.

i) Detergent Dialysis:

A pilot plant under the trade name of LIPOPREPR IICIS is available from Diachema, AG, Switzerland. The production capacity at higher lipid concentration (80 mg/ml) is 30 ml liposomes/minute. But when lipid concentration is 10-20 mg/ml 100 mg/ml then up to many litres of liposomes can be produced. In USA, LIPOPREPR is marketed by Dianorm-Geraete.

ii) Microfluidization:

A method based on microemulsiftcation/homogenization was developed for the preparation of liposomes. MICROFLUIDIZERR is available from Microudics Corporation, Massachusetts, USA. A plot plant based on this technology can produce about 20 gallon/minute of liposomes in 50-200 nm size range. The encapsulation efficiency up to 75% could be obtained.

iii) Aqueous dispersions of liposomes often have tendency to aggregate or fuse and may he susceptible to hydrolysis and or oxidation. Two solutions have been proposed:

(a) Proliposomes:

In proliposomes, lipid and drug are coated onto a soluble carrier to form free-flowing granular material which on hydration forms an isotonic liposomal suspension. The proliposome approach may provide an opportunity for cost-effective large scale manufacture of liposomes containing particularly lipophilic drugs.

(b) Lyophilization:

Freeze-drying (lyophilization) involves the removal of water from products in the frozen state at extremely low pressures. The process is generally used to dry products that are thermolabile and would be destroyed by heat-drying. The technique has a great potential as a method to solve long term stability problems with respect to liposomal stability. It is exposed that leakage of entrapped materials may take place during the process of freeze- drying and on reconstitution. Recently, it was shown that liposomes when freezedried in the presence of adequate amounts of trehalose (a carbohydrate commonly found at high concentrations in organism) retained as much as 100% of their original contents. It shows that trehalose is an excellent cryoprotectant (freeze-protectant) for liposomes.

Factors considered in preparation of liposome:

Raw material and their specifications:

1. lipids from natural sources-egg lecithin can vary in fatty acid composition
2. modified natural lipids
3. semi synthetic lipids
4. synthetic lipids

Manufacturing process and controls:

1. removal of residual organic solvent
2. removal of endotoxin
3. removal of free drug
4. protection of lipids from oxidation
5. control of liposome size distribution/osmolality
6. encapsulation control

7. sterilization
8. scale up and economic feasibility
9. storage and reconstitution
10. liposome’s preparation can be stored frozen in liquid form as freeze dried powder
11. reconstitution of freeze dried liposome’s may affect particle size and size distribution.

((Dr)A.K.Bandyopadhyay n.d.)

Characterization of Liposomes:

A)Biological characterization: (Table 5)

Characterization parameters	**Instrument for analysis**
Sterility	Aerobic/anaerobic culture
Pyrogenicity	Rabbit fever response
Animal toxicity	Monitoring survival rats

(Wiener 1998.)

B)Chemical characterization: (Table 6)

Characterization parameters	**Instrument for analysis**
Phospholipid concentration	HPLC/barlet assay
Cholesterol concentration	HPLC/ Cholesterol oxide assay
Drug concentration	Assay method
Phospholipids per oxidation	UV absorbance
Phospholipids hydrolysis	HPLC/ TLC
Cholesterol auto-oxidation	HPLC/ TLC
Autooxidant degradatio	HPLC/ TLC
pH	pH meter
osmolarity	osmometer

(Wiener 1998.)

C)Physical characterization: (Table 7)

Characterization parameter	**Instrument for analysis**
Vesicle shape and surface morphology	TEM and SEM
Vesicle size and size distibution	Dynamic light scattering,TEM
Surface charge	Free flow electophoresis
Electrical surface potential and surface pH	Zeta potential measurement and PH sensitive probes
Lamellarity	P31 NMR
Phase behaviour	DSC, freeze fracture electronic microscopy
Percent capture	Minicolumn centrifugation,gel exclusion
Drug release	Diffuse cell/dialysis

(Wiener 1998.),

The most important parameters of liposome characterization include visual appearance, turbidity, size distribution, lamellarity, concentration, composition, presence of degradation products, and stability.

1. Visual Appearance:

Liposome suspension can range from translucent to milky, depending on the composition and particle size. If the turbidity has a bluish shade this means that particles in the sample are homogeneous; a flat, gray color indicates that presence of a nonliposomal dispersion and is most likely a disperse inverse hexagonal phase or dispersed microcrystallines. An optical microscope (phase contrast) can detect liposome> 0.3 μm and contamination with larger particles.

2. Determination of Liposomal Size Distribution:

Size distribution is normally measured by dynamic light scattering. This method is reliable for liposomes with relatively homogeneous size distribution. A simple but powerful method is gel exclusion chromatography, in which a truly hydrodynamic radius can be detected. Sephacryl-S100 can separate liposome in size range of 30-300nm. Sepharose -4B and -2B columns can separate SUV from micelles.

3. Determination of Lamillarity:

The lamellarity of liposomes is measured by electron microscopy or by spectroscopic techniques. Most frequently the nuclear magnetic resonance spectrum of liposome is recorded with and without the addition of a paramagnetic agent that shifts or bleaches the signal of the observed nuclei on the outer surface of liposome. Encapsulation efficiency is measured by encapsulating a hydrophilic marker

4. Drug entrapment efficiency:

The entrapment efficiency was calculated by using the formula:

% Entrapment efficiency = Entrapped drug / total drug added X100 . To find out the drug content (drug entrapment) the liposomal suspension was ultra-centrifuged at 5000 rpm for 15 min at 4 oC temperatures by using semi cooling centrifuge to separate the free drug. The free drug was formed at wall of the centrifuge tube and suspended containing liposomes were in suspended stage. The supernatant liquid was collected and centrifuged by maintaining same rpm and temperature for about 30 min which forms a clear solution of supernatant and pellets of liposomes. The unentrapped drug were soaked for about 10 min by using methanol and sonicated for about 10min, which causes breakdown of the vesicles to release the drug and the drug content was estimated by UV spectrophotometrically.

5.Differential scanning calorimetry (DSC):

In this method drug loaded multilamellar liposomes were submitted to DSC analyser (modelTA-60, shimadzu). The DSC was performed on the 5 mg sealed in standard aluminium pans.Thermo grams were obtained at a scanning rate of 20°C / min. Each sample was scanned between 0°C to 200°C. This is used to determine phase transition temperature of phospholipids sample in order to characterise the state of drug.

(Sharma Vijay K aug-oct 2010)

6. In vitro drug release study:

The *in vitro* diffusion studies were carried out by using the Franz diffusion cell which has a diameter of 25 mm and a diffusion area of 4.90 cm2. It contains the reservoir compartment of 22 mL, which was filled with a buffer which contains 20% v / v methanol to maintain sink condition. There is regenerated cellulose acetate membrane which is placed in between the lower cell reservoir and the glass cell top containing the sample and is clinched with a clamp. The whole system was maintained at the temperature of 37°C ± 5°C by using magnetic heater resulting in the membrane surface temperature of 32°C. The sample was placed on the surface of the donor compartment and then for the assay process the 2mL of the samples were received from receptor fluid and replaced with 2mL of fresh solution. The time period to carry out the process depends on the half-life of the drug selected and further dilutions are carried out and measured spectrophotometrically.

7. Quasi-elastic light scattering (QELS): The hydrodynamic mean diameters (MD) of the vesicles and mixed aggregates were determined by quasi-elastic light scattering (QELS) using a N4 Coultronics apparatus. Calculations were made according to the Stokes– Einstein equation assuming the particles to be independent and spherical. The MD values correspond to the average of three measurements with a standard deviation lower than 5%.

(Karine Andrieux (2009)

8. Entrapped Volume:

The entrapped volume of a population of liposome (in µL/ mg phospholipid) can often be deduced from measurements of the total quantity of solute entrapped inside liposome assuring that the concentration of solute in the aqueous medium inside liposomes is the same after separation from unentrapped material. For example, in two phase method of preparation, water can be lost from the internal compartment during the drying down step to remove organic solvent.

9. Surface Charge:

Liposomes are usually prepared using charge imparting constituting lipids and hence it is imparting to study the charge on the vesicle surface. In general two methods are used to assess the charge, namely free flow electrophoresis and zeta potential measurement. From the mobility of the liposomal dispersion in a suitable buffer, the surface charge on the vesicles.

Stability of Liposomes:

In general successful formulation of stable liposomal drug product requires the following precautions

1. Processing with fresh, purified lipids and solvents.
2. Avoidance of high temperature and excessive shear forces
3. Maintenance of low oxygen potential (Nitrogen purging)
4. Use of antioxidant or metal chelators
5. Formulating at neutral pH.

6. Use of lyo-protectant when freeze drying
(Yadav A.V Oct-Dec 2011)

Classification of Stability of Liposomes: Liposome stability can be subdivided into physical, chemical and biological stabilities, which are all inter-related. Generally, the shelf-life stability of liposomes is determined by the physical and chemical stability (Uniformity of size distribution and encapsulation efficiency, and minimal degradation of all compounds, respectively). By optimizing the size distribution, pH and ionic strength, as well as the addition of antioxidants and chelating agents, liquid liposome formulations can be stable.
(Yadav A.V Oct-Dec 2011), (Lasic 1998)

1. Chemical stability:

As phospholipids usually form the backbone of the bilayer their chemical stability is important. Two types of chemical degradation reactions can affect the performance of phospholipid bilayers: hydrolysis of the ester bonds linking the fatty acids to the glycerol backbone and peroxidation of unsaturated acyl chains (if present). The oxidation and hydrolysis of lipids may lead to the appearance of short-chain lipids and then soluble derivatives will form in the membrane, resulting in the decrease of the quality of liposome products.
(Yadav A.V Oct-Dec 2011), (Chen and Alli 1987)

2. Physical stability:

Physical processes such as aggregation/flocculation and fusion/coalescence that affect the shelf life of liposomes can result in loss of liposome associated drug and changes in size. Aggregation is the formation of larger units of liposomal material; these units are still composed of individual liposomes. In principle, this process is reversible e.g. by applying mild shears forces, by changing the temperature or by binding metal ions that initially induced aggregation. However, the presence of aggregation can accelerate the process of coalescence of liposomes, which indicates that new colloidal structures are formed.As coalescence is an irreversible process; the original liposomes cannot be retrieved.

A colloidal dispersion is often thermodynamically unstable. Spontaneous processes occur in the direction of decreasing Gibbs free energy; therefore the separation of a two-phase dispersed system to form two distinct layers is a change in the direction of decreasing Gibbs free energy. There is more surface energy in a liposome suspension when the dispersed phase is in a highly subdivided state than when it is in a coarser state of subdivision. The central feature of coalescence is the fact that the total surface area is reduced in the coarsening process of thermodynamically unstable liposome dispersion, while there is no reduction of surface in aggregation, although certain surface sites may be blocked at the points at which the smaller particles touch13. After small particles coalescence, only the new larger particle remains. With aggregation, however, the small particles retain their identity and the aggregation moves as a single unit.
(Yadav A.V Oct-Dec 2011)

3. Biological stability:

Biological stability of liposomes, however, depends on the presence of agents such as proteins that interact with liposomes upon application to the subject and depends on the administration route. Strategies used to enhance biological stability of liposomes will improve liposome-mediated drug delivery in vivo and increase circulation time in the blood stream. It was observed that aerosols of interleukin 2 liposomes were biologically stable and retained in the lung after nebulization15. Incorporating steric stability, e.g. the incorporation of N-acyl-phosphatidyl ethanolamine into liposomes has shown to increase the liposomal biological stability towards plasma components.
(Yadav A.V Oct-Dec 2011)

Methods for enhancement of stability:

Maximum encapsulation efficiency and absence of drug leakage i.e. drug retention are the major goals in vesicular drug delivery system. Achieving such goals is possible only if the physical, chemical biological or in vitro / in vivo drug retention stability of liposomes is enhanced. Drug retention stability depends on the number of factors like, method of manufacture, method of drug loading, lipid composition, physicochemical properties of drug, In present review we tried to explain methods collectively because these are all inter-related.

1. Control of particle size and lamellarity*:*

Any change in particle size can affect targeting, RES uptake, safety and efficacy. Liposomes are classified according to the size and lamelliarity. Aggregation and fusion is mostly observed with particle size < 20 nm that have excessive high stress curvature which promotes this phenomenon. Fusion can be prevented by good manufacturing procedure.Small unilamellar vesicles (SUV), 25 to 100 nm in size that consists of a single lipid bilayer. Large unilamellar vesicles (LUV), 100 to 400 nm in size that consists of a single lipid bilayer.Multilamellar vesicles (MLV), 200 nm to several microns that consist of two or more concentric bilayers. Vesicles above 1 µm are known as giant vesicles. Particle size and lamellarity depends on the method of manufacture. The lamellarity of liposomes influences to a great extent the encapsulation efficiency, the efflux rate of liposomal encapsulated material, and the fate of a drug after cellular uptake.

(Yadav A.V Oct-Dec 2011)

2. Lipid composition:

Permeability and stability of liposomes are influenced by the rigidity/stiffness of the lipid bilayer. Selection of lipid in turn depends on the phase transition temperature of lipids, which depends on the acyl chain length. Gel-liquid phase transition occurs in a narrow temperature range for pure lipids (Tc). Tc is affected by fatty acid side chains, degree of unsaturation, chain length and polar head groups. Lipids with long acyl chain length are most commonly used because high phase transition temperature. All these things are not possible by using single lipid; this can be achieved by combinations of lipids or incorporation of another substances. Temperature affects Phosphatidyl choline (PC) liposomes stability and the optical densities of these liposomes were increased with time (days), while incorporation of cholesterol 25mol % to the liposomes (PC: cholesterol) reduce the temperature's effects and the optical densities show a very slight change with time (days) because cholesterol in high concentrations prevent the phospholipids packing and induce orientation and more rigidity to those phospholipids and therefore prevent liposome aggregation, liposmes prepared by using combinations of some lipids follows the order of physical stability form the correlation of the mean volume diameter, zeta potential and pH , egg lecithin (PC)/cholesterol (CH)/stearylamine (SA) < PC/CH/phosphatidylserine (PS) < bovine brain ceramides (CM)/CH/palmitic acid (PA)/CS < PC/CH/cholesteryl sulphate (CS) at 4°C, as well as at 25°C, after a 6-month storage period. Chol (cholesterol) addition in lipid membrane improves PC liposome integrity, but has opposite or no effect on liposomes consisting of saturated lipids. Charge on the liposomes also determines the in vivo stability. Liposomes with neutral charge containing phosphatidylcholine were the most stable and bound the lowest amount of protein. Liposomes with positive charge behaved similarly to those with neutral charge. However, the stability of negatively charged liposomes was very dependent on their composition. Those liposomes containing only one class of negatively charged phospholipids bound a great amount of protein and were very unstable. However, those liposomes containing phosphatidylcholine bound less protein and were more stable**.**

(Yadav A.V Oct-Dec 2011)

3. Method of drug loading:

Drug loading methods involves passive and active loading. Passive loading includes mechanical dispersion, solvent dispersion and detergent solubilisation working on different principles. Remote or active loading method load drug molecules into preformed vesicles by using pH gradient and potential difference across liposomal membranes. The stability of the drug encapsulation was measured by various assays and it was found that according to the theoretical predictions, active loading give the greater encapsulation efficiency. One which invention provides the encapsulation of weak acid compounds includes ibuprofen, tolmetin, indomethacin, phenylbutazone, meclofenamic acid, piroxicam, ciprofloxacin, and nalidixic acid. Invention provides a method for loading a weak-acid compound into liposomes having a higher inside/lower outside pH gradient. Loading is carried out by adding the weak acid compound to a suspension of liposomes having a higher inside/lower outside gradient of a salt of a weak acid which includes the given cation. The protonated form of the weak acid salt acts as an inside-to-outside proton shuttle to generate a higher inside/lower outside pH gradient to drive loading of the weak acid compound into the liposome interior. In the absence of cholesterol, liposome permeability is enhanced at these temperatures which, in turn, can result in the collapse of the pH gradient and/or unstable loading. Ethanol-induced increased drug loading was temperature-, lipid composition and lipid concentration-dependent. Ethanol addition to preformed liposomes is an effective method to achieve efficient pH gradient-dependent loading of cholesterol-free liposomes at temperatures below the *T*c of the bulk phospholipid retention.

(Yadav A.V Oct-Dec 2011)

Prodrug:

Physical properties of drug play a role in the stability and hence drug delivery kinetics of liposomal drug delivery system. Prodrug is another approach to enhance the drug retention stability. Problems like poor entrapment efficiency and physical as well as chemical instability have been found to be associated with the liposomal entrapment of drug molecules, other than those that are highly lipophilic. Prodrug lipophilic character improves the interaction with lipid bilayers, favouring the absorption through the lipid barriers by allowing liposomes to work as a lipophilic carrier which is able to deliver drug near to the cell surface. Only care we have to take is that hydrophobic acyl chains anchored to the drug must match to acyl chains of lipids. Water-soluble antineoplastic agent 5-fluro-2'-deoxyuridine (FUdR) encapsulated in water phase. Retention was assessed with plasma, storage upon refrigeration, diffusion of FUdR across liposomes faster in fluid type liposomes than lipophilic prodrug FUdR-dipalmitate and FUdR-dioctanoate.

(Yadav A.V Oct-Dec 2011)

5. Pro-liposomes:

In dispersed aqueous system liposomes have a problem of degradation by hydrolysis or oxidation as well as sedimentation, aggregation, or fusion of liposomes during storage Other problem associated with clinical application of liposomes includes difficulties in large-scale production to obtain a product with adequate physical and chemical stability. In formulation of proliposomes lipid dried over a fine particulate, water soluble support like sodium chloride, sorbitol or polysaccharides imparts adequate physical and chemical stability and are ideally suitable for lipophilic drugs.

(Yadav A.V Oct-Dec 2011)

6. Lyophilization:

The problems related to lipid oxidation and hydrolysis during shelf life of the liposomal product can be reduced by the storage of liposomal dispersion in the dry state. Changes in the arrangement of phospholipids within the bilayer of small vesicles have been attributed to the loosely packed head groups and tightly packed alkyl chains in the outer layer with the opposite arrangement in the inner layer of the bilayer.This leads to a thermodynamically unstable state, which favors aggregation and/or fusion of the vesicles to counteract this instability. Therefore, it is clear that the composition of the lipid bilayer and the aqueous phase, the amount of external water, bilayer–drug interaction, and the storage conditions of the liposomal formulations will all influence liposome stability. To circumvent these problems and achieve long-term stability of liposome’s, freeze-drying (Lyophilization) has been used as an effective approach to render liposomes stable without compromising their physical state or encapsulation capacity. However, freeze-drying of liposome systems without appropriate stabilizers will again lead to fusion of vesicles; a factor exploited by the dehydration–rehydration method.

To promote vesicle stability during the freeze-drying process, cryoprotectants, including saccharides (e.g. sucrose and lactose) and their derivatives, are employed briefly; the increase in vesicle size and leakage of the encapsulated material in non-cryoprotected liposomes during freeze-drying is attributed to two factors: phase separation of lipids around their gel–fluid transition temperature and fusion of the membranes of the liposomes. Thus, aggregation of liposomes could be prevented by the formation of stable boundaries between the vesicles. The ability of cryoprotectants to form these stable boundaries has been attributed to their ability to replace the bound water around the bilayer via interaction with the polar region of the lipid head group. The liposomal dispersions could be stored at room temperature in the freeze-dried form with minimum effect on stability, drug content and physicochemical properties. The freeze-dried lyophilized powder could be used for incorporation of liposomes in topical gel formulation (in powder form or reconstituted dispersion form) or could be reconstituted immediately prior to injection or further formulation processing. (Yadav A.V Oct-Dec 2011)

7. Electrosteric stabilization:

The first description of sterically stabilized liposomes was reported in 1987-1988. Stable liposome suspensions require a repulsive interaction that is at least comparable to the magnitude and range of the Vander Waals force. The electrostatic repulsion can be realized by increasing the charge of a surface by lowering the ionic strength or altering the pH away from the point of charge of the solid or addition of charged molecules in the bilayer, which shift the electrophoretic mobility. A (very big) disadvantage of electrostatic stabilization is that it is highly sensitive with respect to surface charge (pH) and salt concentration. Another way, steric stabilization can be achieved by covering the surface with an adsorbed layer of long, bulky molecules to prevent the close approach of the liposomes. The combination of electrostatic and steric stabilization, which has been termed electrosteric stabilization, leads to highly stable dispersions. The electrostatic component results from a net charge on the liposome surface and/or the charge associated with the adsorbed polymer. The steric component arises from the chain-chain exclusion of adsorbed polymeric material on one surface from the other surface. Addition of other interactions expands the DLVO model to an extended DLVO inter-particlse potential. For the most general case, the total potential between liposome's should include all the forces, including van de Waals attraction (V_{vdW}), electrostatic repulsion (V_{ele}), hydrophobic attraction (V_{hfo}), hydration (V_{hyd}), undulation (V_{und}), steric repulsion (V_{st}), repulsive protrusion forces (V_{pr}), and attractive ion correlation forces (V_{icf})

$V_{tot}=(V_{vdW})-(V_{hfo})-(V_{icf})+(V_{ele})+(V_{hyd})+(V_{und})+(V_{st})+(V_{pr})$

Liposomes bound or coated with ligands, such as PEG,Carbopol1342,polyvinylalcohol, o-palmitoylpullulan, poloxamer338,poly(vinylpyrrolidone), dicetyl phosphate (negative charge), stearylamine (positive charge)carboxymethylchitin or block copolymers have been investigated and shown to increase the liposome stability and control the drug release.

PEG is the most widely used stabilizer in pharmaceutical applications because of its advantages including non-biodegradability, high solubility in aqueous solution, ability to bind a large number of water molecules, high flexibility of its polymer chain, immunogenicity, antigenicity and limited accumulation in cells of the reticular endothelial system (RES). In addition, it has been shown that PEG could prevent the adsorption of proteins (opsonins) responsible for phagocytic removal, and provides the ability to independently adjust the liposome physicochemical properties (such as drug loading and leakage) and biological properties (e.g. blood circulation and tissue distribution). The term 'steric protection' is used with regard to liposome interaction with 'opsonins'. We should note that the term 'steric protection' is not self –explanatory. The 'steric' is as vague as "having to do with spatial arrangement if the atoms in the molecule."

(Yadav A.V Oct-Dec 2011)

Marketed formulations: **(Table 8)**

Product	Drug	Company
Ambisome^{TM}	Amphotericin B	NeXstar Pharmaceuticals, Inc., CO
Abelcet^{TM}	Amphotericin B	The Liposome Company, NJ
Amphocil^{TM}	Amphotericin B	Sequus Pharmaceuticals, Inc., C.A.
Doxil^{TM}	Doxorubicin	Sequus Pharmaceuticals, Inc., C.A.
DaunoXome^{TM}	Daunorubicin	NeXstar Pharmaceuti

		cals, Inc., CO
MiKasome^{TM}	Amikacin	NeXstar Pharmaceuticals, Inc., CO
DC99^{TM}	Doxorubicin	Liposome Co., NJ, USA
Epaxel^{TM}	Hepatitis A Vaccine	Swiss Serum Institute, Switzerland

(Sharma Vijay K1* aug-oct 2010)

Applications:

1) Liposomes in the sciences: (Table 9)

Discipline	Application
Mathematics	Topology of two-dimensional surfaces in three-dimensional space governed only by bilayer elasticity
Physics	Aggregation behaviour, fractals, soft and high-strength materials
Biophysics	Permeability, phase transitions in two-dimensions, photophysics
Physical Chemistry	Colloid behaviour in a system of well-defined physical characteristics, interand intra-aggregate forces, DLVO
Chemistry	Photochemistry, artificial photosynthesis, catalysis, microcompartmentalization
Biochemistry	Reconstitution of membrane proteins into artificial membranes
Biology	Model biological membranes, cell function, fusion, recognition
Pharmaceutics	Studies of drug action
Medicine	Drug-delivery and medical diagnostics, gene therapy

(RIAZ July 1995), (Abdus Samad 2007)

2) Liposomes in the pharmaceutical industry: (Table 10)

Liposome Utility	Current Applications	Disease States Treated
Solubilization	Amphotericin B, minoxidil	Fungal infections
Site-Avoidance	Amphotericin B – reduced nephrotoxicity,	Fungal infections, cancer
Drug protection,	Cytosine arabinoside, interleukins	Cancer, etc.
RES Targeting,	Immunomodulators, vaccines, antimalarials, macophage-located diseases	Cancer, MAI, tropical parasites
Specific Targeting	Cells bearing specific antigens	Wide therapeutic applicability
Extravasation	Leaky vasculature of tumours, inflammations, infections	Cancer, bacterial infections
Accumulation	Prostaglandins	Cardiovascular diseases
Enhanced Penetration	Topical vehicles	Dermatology
Drug Depot	Lungs,subcutaneous, intra-muscular, ocular	Wide therapeutic applicability

(Abdus Samad 2007)

Other Applications:

1. Treatment of Diseases:

Drug containing liposomes have been shown to be effective against diseases in test animals and in some cases in human beings. (RIAZ July 1995),

2. as Vaccine Carriers:

Liposomes carrying antigens (derived from Infectious organisms) particularly viral antigens, malaria antigens and bacterial toxins have been successfully used to produce humoral or cellular immunity in test animals. This indicates that liposomes have potential as vaccine carriers.

(RIAZ July 1995),

(Table 11) Some Antigens as Liposomal Preparation and their Applications:

Antigens as Liposomal Preparation	**Applications**
Rabies glycoproteins	Interleukin -2 enhancement
Cholera toxin	Enhanced Ab* level
Diphtheria toxoid	Superior immunoadjuvant
Herpes simplex virus	Enhanced Ab level
Hepatitis B virus	Higher Ab response
Bacterial polysaccharides	Superior immunoadjuvants
Tetanus toxoids	Increased Ab titre
Influenza subunit antigen	Intranasal, protects animal from virus
Carbohydrate antigen	Increased Ab titre in salivary gland

3. In Cell Physiology:

Liposomes can be constituted so that cell membranes are functionally expressed on the surface of liposomes, thereby providing a model system for studying receptor membrane interactions.

As Diagnostic Agents:

The intravenous administration of liposomes containing contrast agents such as 99m Tc have been used to visualize certain malignant tissues such as cancerous tissues of breast, rheumatoid factor, etc.

4. **As Model Membranes:**

i) Liposomes have been used successfully to investigate the mechanism involved in the ototoxicity of aminglycosid antibiotics. On the basis of these studies, the following multistep mechanism was proposed:

(1) Interaction of aminoglycoside with phospholipids in the outer plasma membranes.

(2) Aminoglycoside translocation into cell and Binding of the drug to phosphatidylinositol 4, 5 diphosphate. Further these studies have shown that the binding of PIP2 containing liposomes to aminoglycosides is in accordance with the established toxicity of aminoglycosides: Neomycin > gentamicin > amikacin > neamine.

ii) Liposomes have been used to understand the mode of action of local anesthetics. These studies have indicated that the action of a local anesthetic is mediated by the specific interaction with phosphatidylserine. These interactions probably cause significant membrane fluidization and displacement of Ca2+ from membranes. These two effects are also associated with anesthetic action of excitable membranes.

iii) Liposomes appear to be convenient in vitro models for studying the phototoxic effects mediated through active oxygen species, membrane damage and altered Ca fluxes.

(RIAZ July 1995)

5. In Cosmetics:

To investigate the use of liposomes in cosmetics, studies have been made to study interaction between skin and liposomes.

According to the manufacturers, liposomes may deliver moisture and a novel supply of lipid molecules to skin tissue in a superior fashion to other formulations. In addition they can entrap a variety of active molecules and can therefore be utilized for skin creams, antiaging creams, after shave, lipstick, sun screen and make-up.

(RIAZ July 1995),

6. In Textiles: A new method of wool chlorination at pH < 7 using liposomes as vehicles for oxidizing agents is suitable for inhibiting or modulating the formation of cystic acid in wool fibers.The chlorination using liposomes inhibits the formation of cystic acid. (RIAZ July 1995)

7. Liposome in Tumour Therapy

The long term therapy of anticancer drug leads to several toxic side effect. The liposomal therapy for the targeting to the tumour cell have been revolutionized the world of cancer therapy with least side effect. It has been said that the small and stable liposome are passively targeted to different tumour because they can circulate for longer time and they can extra vasate in tissue with enhanced vascular permeability.Liposome macrophage uptake by liver and spleen hampered the development of liposome as drug delivery.

(Table 12) Various Intravenous Liposomal Antibiotics / Anti-Neoplastics:

Preparation	Drug	Targeted Site
Liposome (Doxil)	Doxorubicin	Kaposi' sarcoma
Liposome (EVACTTM)	Doxorubicin	Refractory tumour Metastatic breast cancer
Liposome (DaunoXome)	Daunosome	Advanced Kaposi' sarcoma, breast , small cell lung cancer, leukaemia and solid tumour
Liposome	Nystatin	Systemic fungal infection
Liposome	Anamycin	Kaposi' sarcoma, Refractory breast cancer
Liposome (VincaXome)	Vincristine	Solid tumour

(Abdus Samad 2007)

8. Liposome for Respiratory Drug Delivery System:

Liposome is widely used in several types of respiratory disorders.

Liposomal aerosol has several advantages over ordinary aerosol which are as follows:

1. Sustained release
2. Prevention of local irritation
3. Reduced toxicity and
4. Improved stability in the large aqueous core.

(Table 13) Liposomal Formulation for the Respiratory Disorder:

Active constituent	Effect
Insulin	Facilitated pulmonary adsorption and enhanced hypoglycemic effect
Catalase	Conferred resistance to pulmonary oxygen toxicity
Super oxide dusmutase	Minimize toxicity to subsequent hyperoxia and improved survival
Cyclosporins	Preferentially adsorbed by lung and shows sustained release
Ricin vaccine	Improved safety profile for intra pulmonary vaccination
Interleukin-	2 The lungs Facilitated bioactivity and reduce toxicity
Isoniazid and Rifampicin	Improved the effect of drugs for the tuberculosis

(Abdus Samad 2007)

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PARTICULATE DRUG DELIVERY SYSTEMS – MICROCAPSULES

Sawant Sandip, Pawar Yogesh

INTRODUCTION:

During the past decades, there has been an increasing interest in optimizing the efficiency of existing drugs through the use of better-designed drug delivery systems. Intensive interdisciplinary research efforts have led to a variety of advanced dosage forms. The majority of these systems are based on polymers that differ in their permeability, rate of dissolution, degree of swelling and erodibility. An important class of polymer mediated drug delivery systems that are applied for controlled drug delivery is the microcapsules (SACHAN N.K. * 2006). Microencapsulation provides the means of converting liquids to solids, of altering colloidal and surface properties, of providing environmental protection and of controlling the release characteristics or availability of coated materials. Several of these properties can be attained by macro packaging techniques; however, the uniqueness of microencapsulation is the smallness of the coated particles and their subsequent use and adaptation to a wide variety of dosage forms and not has been technically feasible (Chowdhury R. S.* 2012) (Bansode S.S* 2010). Firstly the microencapsulation procedure was discovered by Bungen burg de Jon and Kan in 1931and which were deal with the preparation of gelatin spheres and use of a gelatin coacervation process (Kumar A* 2011). Microcapsules according to the French Pharmacopoeia are solid material consisting of a solid envelope containing a liquid or solid or a pasty substance. The microcapsules occur in the form of powder with particles less than 1250 µm in diameter (SACHAN N.K. * 2006).

Novel drug delivery systems which were initiate with the course of optimizing the bioavailability by the modification of the bioavailability of the drug concentration in blood. With the sustained and controlled release products, drug therapy can be improved that is the common goal achieved over with their non sustained and controlled release with the same drug. Microencapsulated products (micro Particles) are the small entities that have an active agent know as the core material surrounded by a shell known as the coating material or embedded into a matrix structure. Most Microparticle shells are of organic polymers, but waxes and lipids are also used. The ingredients to be coated are referred to as core, internal phase (IP), encapsulate or fill, whereas terms applied to the coating of the microcapsules include the wall, shell, external phase or membrane. All three states i:e. solid, liquid and gases, may be encapsulated and affect the size and shape of the capsules. If a solid or a crystalline material is used as the core, the resultant capsule may be irregularly shaped. However, if the core material is a liquid, simple spherical capsules, containing a single droplet of encapsulate, may be formed (Chowdhury R. S.* 2012). Generally the size of the microencapsulated products (microparticles) is considered as larger than 1micrometer and up to 1000 micrometers in diameter. Commercially available microparticles contained 10-90% w/w core. A number of core materials can be encapsulated like that live cells, adhesives, flavors, agrochemicals, enzymes, pharmaceuticals. The more recent result of pharmaceutical research is that the absorption rate of a drug can be controlled by controlling its rate of release from the dosage form .The controlled released dosage forms are so designed and formulated as having the sustained action, sustained release, prolonged action, delayed action and timed release medication. This has been done by developing the new drug entities, discovering of new polymeric materials that are suitable for prolonging the drug release, safety, improvement in therapeutic efficacy (Kumar A* 2011) (Chowdhury R. S.* 2012). The scanning electron microscopy (SEM) is used to reveal the structural features of microcapsules as these are to be vary and complex. The walled prototype may be mononuclear as shown in Figure1(a) or may have multiple core structure. And there may also be double or multiple concentric coating present. As aggregated microcapsules have an additional external wall and thus very in shape and size Fig1(b). Although, the microstructure of membrane and interior can be detected by SEM of surfaces or sections Fig1(c) and their physical quality is not easily characterized quantitatively in microcapsules as involving the measurements of porosity, tortuousity and crystallinity, but there is some progress has been made and efforts are to calculate porosity and permeability from release data, densities, dimensions, and core/wall ratios (SACHAN N.K. * 2006) (Kumar A* 2011).

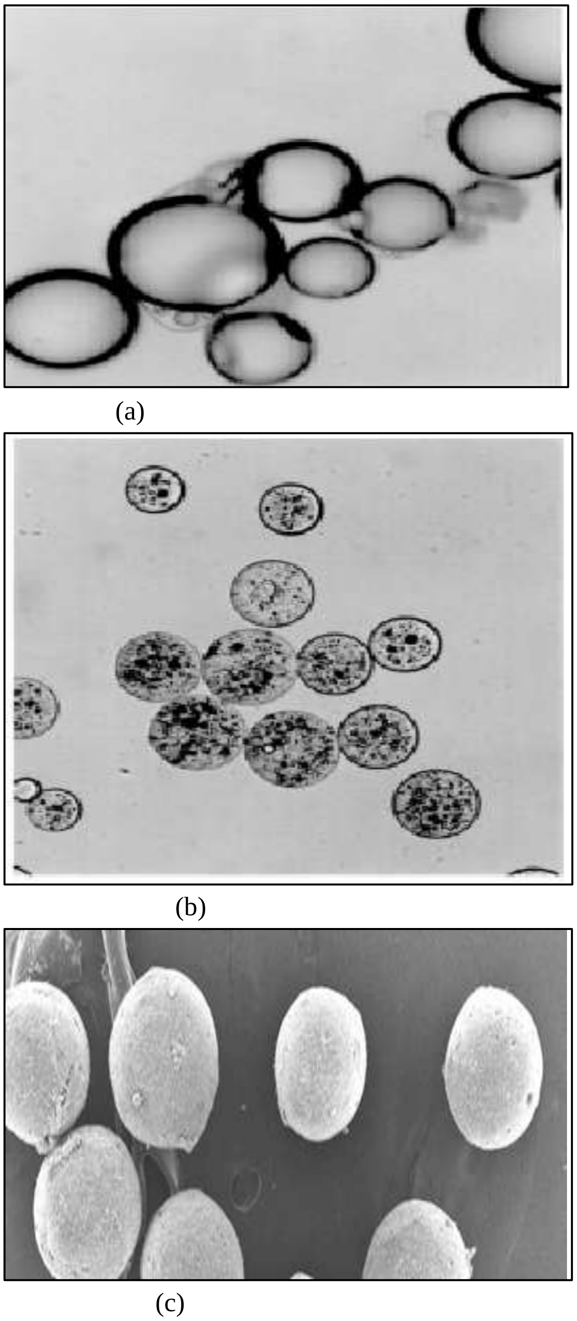


Fig. 1: Scanning electron micrographs (×100) of (a) mononuclear, (b) multinuclear microspheres, and (c) surface of the microspheres.

The standard pharmaceutical dosage forms are employed, like that hard gelatin capsules, which also may be enteric coated, soft gelatin capsules, or suspensions in liquids, all of these allow dispersion of individual microcapsules on release (Chowdhury R. S.* 2012) (SACHAN N.K. * 2006). There has been some recent interest in tableted microcapsules, which will however, only restore the original microcapsules if suitably formulated to undergo complete disintegration. Several publications deals with non-disintegrating tablets, intended to extend release time particularly of soluble drugs and in fact doing so, but to greatly varying extent. The release is affected by many factors (excipients, compaction pressure, and usual tableting factors). The advantage of using costlier microcapsules as opposed to conventional mixtures of drug and polymer in sustained release matrix type tablets is no means clear, except when microencap-sulation offer additional benefits (SACHAN N.K. * 2006).

Reasons for Microencapsulation:

1. The main reason for microencapsulation is for sustained or prolonged release of the drug.
2. This technique has been widely used for masking the organoleptic properties like taste and odor of many drugs and thus improves patient compliance e.g. Paracetamol, nitrofurantoine for masking the bitter taste.
3. By using microencapsulation techniques the liquid drugs can be converted in a free flowing powder.
4. The drugs can be protected by microencapsulation which is sensitive to moisture light and oxygen, such as nifedipine is protected from photo instability.
5. Microencapsulation technique also helpful to prevent the incompability between drugs
6. The drugs which are volatile in nature may vaporize at room temperature like Aspirin and peppermint oil can be prevented by microencapsulation.
7. Reduction in toxicity and GI irritation including with KCL and ferrous sulphate can be achieved by microencapsulation
8. Microencapsulation has also been employed to change the site of absorption. This application has

been useful for those drugs which have the toxicity at lower pH.

9. Bakan and Anderson were reported that microencapsulated vitamin A palmitate had enhanced stability, as prevent from oxidation.

10. Microencapsulation method has also been employed to prepare intrauterine contraceptive device (Chowdhury R. S.* 2012) (Kumar A* 2011).

Material For Microencapsulation:

CORE MATERIALS:

The core material, defined as the specific material to be coated, can be liquid or solid in nature. The composition of the core material can be varied, as the liquid core can include dispersed and/or dissolved materials. The solid core be active constituents, stabilizers, diluents, excipients, and release-rate retardants or accelerators. The ability to vary the core material composition provides definite flexibility and utilization of these characteristics often allows effectual design and development of the desired microcapsule properties.

COATING MATERIALS:

The coating material should be capable of forming a film that is cohesive with the core material; be chemically compatible and nonreactive with the core material; and provide the desired coating properties, such as strength, flexibility, impermeability, optical properties, and stability. The coating materials used in microencapsulation methods are amenable, to some extent, to in situ modification. The selection of a given coating often can be aided by the review of existing literature and by the study of free or cast films, although practical use of free-film information often is impeded for the following reasons:-

1. Cast or free films prepared by the usual casting techniques yield films that are considerably thicker than those produced by the microencapsulation of small particles; hence, the results obtained from the cast films may not be extrapolate to the thin microcapsule coatings.
2. The particular microencapsulation method employed for the deposition of a given coating produces specific and inherent properties that are difficult to simulate with existing film-casting methods.
3. The coating substrate of core material may have a decisive effect on coating properties. Hence, the selection of a particular coating material involves consideration of both classic free-film data and applied results.

COATING MATERIAL PROPERTIES:

1. Stabilization of core material.
2. Inert toward active ingredients.
3. Controlled release under specific conditions.
4. Film-forming, pliable, tasteless, stable.
5. Non-hygroscopic, no high viscosity, economical.
6. Soluble in an aqueous media or solvent, or melting.
7. The coating can be flexible, brittle, hard, thin etc.

Examples of coating materials:

1. Water soluble resins – Gelatin, Gum Arabic, Starch, Polyvinylpyrrolidone, Carboxymethylcellulose, Hydroxyethylcellulose, Methylcellulose, Arabinogalactan, Polyvinyl alcohol, Polyacrylic acid.
2. Water insoluble resins – Ethylcellulose, Polyethylene, Polymethacrylate, Polyamide (Nylon), Poly (Ethylene- Vinyl acetate), and Cellulose nitrate, Silicones, Poly (lactideco- glycolide).
3. Waxes and lipids – Paraffin, Carnauba, Spermaceti, Beeswax, Stearic acid, Stearyl alcohol, Glyceryl stearates.
4. Enteric resins – Shellac, Cellulose acetate phthalate, Zein (Bansode S.S* 2010) (Chowdhury R. S.* 2012).

Mechanism and Kinetics of Drug Release:

Major mechanisms of drug release from microcapsules include diffusion, dissolution, osmosis and erosion.

1. Diffusion: It is the most commonly involved mechanism wherein the dissolution fluid penetrates the shell, dissolves the core and leak out through the interstitial channels or pores.Thus, the overall release depends on, (1) the rate at which dissolution fluid penetrates the wall of microcapsules, (2) the rate at which drug dissolves in the dissolution fluid, and (3) the rate at which the dissolved drug leak out and disperse from the surface. The kinetics of such drug release obeys Higuchi’s equation (Higuchi 1963) as below:

$Q = [D/J\ (2A - \varepsilon C_S)\ C_S\, t]^{½}$

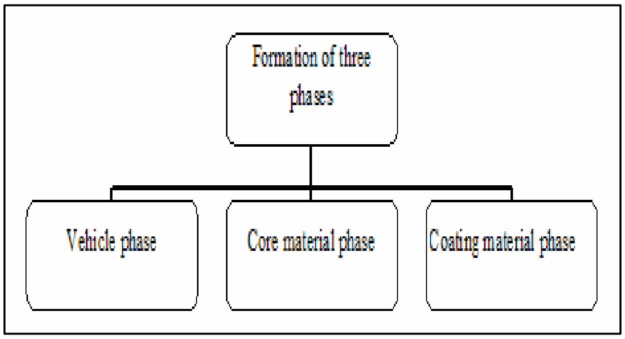


Figure 2: Formation of three phases

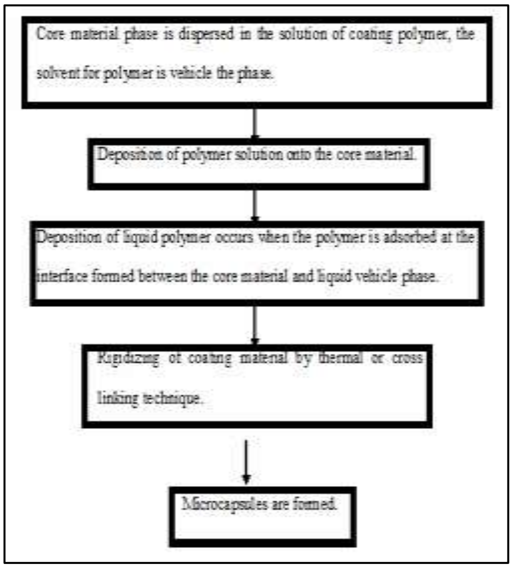

Core material phase is dispersed in the solution of coating polymer, the solvent for polymer is vehicle the phase.
Deposition of polymer solution onto the core material.
Deposition of liquid polymer occurs when the polymer is adsorbed at the interface formed between the core material and liquid vehicle phase.
Rigidizing of coating material by thermal or cross linking technique.
Microcapsules are formed.

Fig no.3 the diagram of formation of three phase in coacervation method

The coacervation-phase separation has been classified into two categories, simple coacervation and complex coacervation. The former implies addition of a strongly hydrophilic substance to a solution of colloid. This added substance causes two phases to be formed [Fig. 4(a)]. The complex coacervation is principally a pH dependant process. The acidic or basic nature of the system is manipulated to produce microcapsules. Above a certain critical pH value, the system depending upon its acidic or basic nature may produce microcapsules. Below that pH value they will not form.Usually complex coacervation deals with the system containing more than one colloid [Fig. 4(b)] (SACHAN N.K. * 2006).

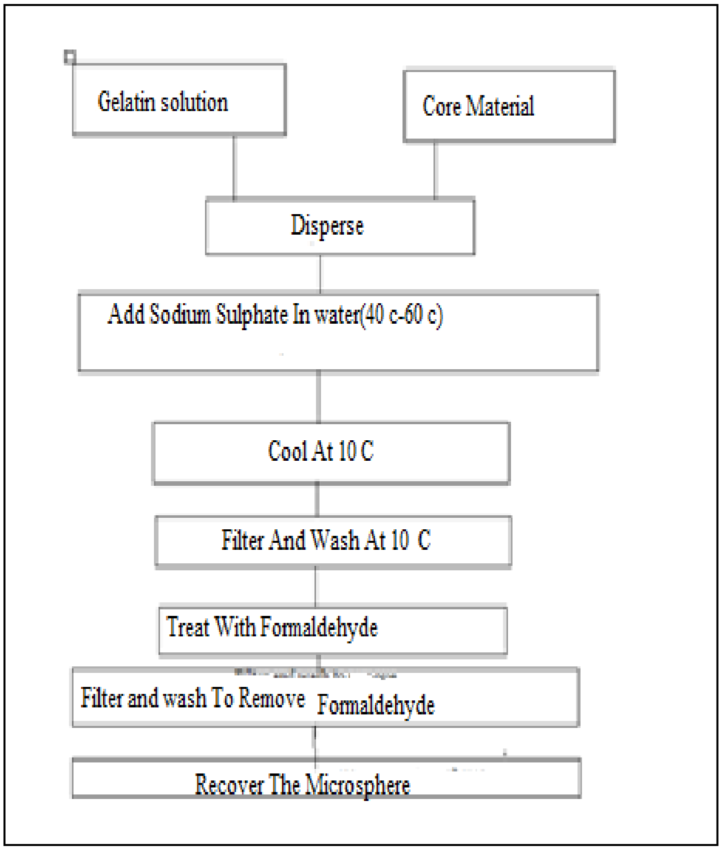
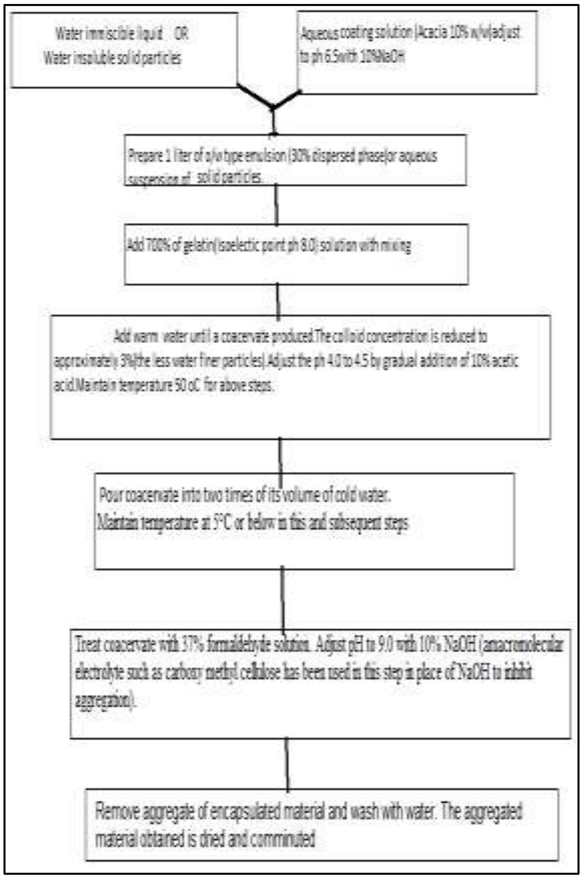


Figure 4(a): Microencapsulation by simple coacervationc.

c. Multi orifice-Centrifugal process:

Microencapsulation by the multi orifice-centrifugal process is the mechanical process in which the centrifugal force is applied to throw a core material particle through an enveloping microencapsulation membrane. The factors affect the Process include the rotational speed of the cylinder, the flow rate of the coating and core materials and the concentration, viscosity and surface tension of the core material. This process is capable of producing the microencapsulation of the liquids and solids of varied size ranges, by applying the different coating materials. The encapsulated product can be supplied as slurry in the hardening media. With this process the production rate of 50 to 75 pounds per hour have been achieved (Chowdhury R. S.* 2012)



Water immiscible liquid OR Water insoluble solid particles

Aqueous coating solution (Acacia 10% w/w)adjust to ph 6.5with 10%NaOH

Prepare 1 liter of o/w type emulsion (30% dispersed phase)or aqueous suspension of solid particles

Add 700% of gelatin(isoelectic point ph 8.0) solution with mixing

Add warm water until a coacervate produced.The colloid concentration is reduced to approximately 3%(the less water finer particles).Adjust the ph 4.0 to 4.5 by gradual addition of 10% acetic acid.Maintain temperature 50 oC for above steps.

Pour coacervate into two times of its volume of cold water. Maintain temperature at 5°C or below in this and subsequent steps

Treat coacervate with 37% formaldehyde solution. Adjust pH to 9.0 with 10% NaOH (amacromolecular electrolyte such as carboxy methyl cellulose has been used in this step in place of NaOH to inhibit aggregation).

Remove aggregate of encapsulated material and wash with water. The aggregated material obtained is dried and comminuted

Fig. 4(b): Microencapsulation by complex coacervation

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d. Pan coating:

The pan coating process, widely used in the pharmaceutical industry, is among the oldest industrial procedures for forming small, coated particles or tablets. The particles are tumbled in a pan or other device while the coating material is applied slowly. The pan coating process, widely used in the pharmaceutical industry, is among the oldest industrial procedures for forming small, coated particles or tablets. The particles are tumbled in a pan or other device while the coating material is applied slowly with respect to microencapsulation, solid particles greater than 600 microns in size are generally considered essential for effective coating, and the process has been extensively employed for the preparation of controlled - release beads. Medicaments are usually coated onto various spherical substrates such as nonpareil sugar seeds, and then coated with protective layers of various polymers. In practice, the coating is applied as a solution, or as an atomized spray, to the desired solid core material in the coating pans. Usually, to remove the coating solvent, warm air is passed over the coated materials as the coatings are being applied in the coating pans. In some cases, final solvent removal is accomplished in a drying oven. The representation of pan coating are present in fig no.5 (Chowdhury R. S.* 2012).

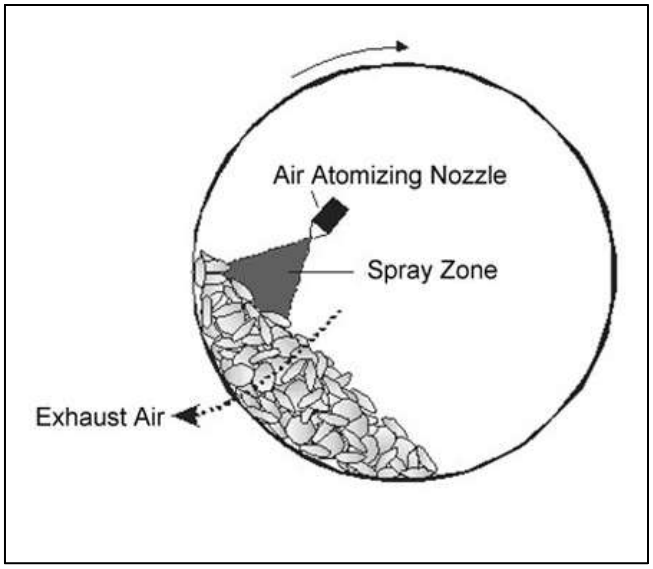

Figure 5: Representation of pan coating

e. Spray-drying And Spray-congealing:

Spray drying serves as a microencapsulation technique when an active material is dissolved or suspended in a melt or polymer solution and becomes trapped in the dried particle. The main advantages is the ability to handle labile materials because of the short contact time in the dryer, in addition, the operation is economical. In modern spray dryers the viscosity of the solutions to be sprayed can be as high as 300mPa.s

Spray drying and spray congealing processes are similar in that both involve dispersing the core material in a liquefied coating substance and spraying or introducing the core-coating mixture into some environmental condition, whereby, relatively rapid solidification (and formation) of the coating is affected. The principal difference between the two methods is the means by which coating solidification is accomplished. Coating solidification in the case of spray drying is effected by rapid evaporation of a solvent in which the coating material is dissolved. Coating solidification in spray congealing methods, however, is accomplished by thermally congealing a molten coating material or by solidifying a dissolved coating by introducing the coating -core material mixture into a nonsolvent. Removal of the nonsolvent or solvent from the coated product is then accomplished by sorption, extraction, or evaporation techniques. List of variables that affecting Spray Drying are listed below in figure 6 (Chowdhury R. S.* 2012) (Bansode S.S* 2010).

In practice, microencapsulation by spray drying is conducted by dispersing a core material in a coating solution, in which the coating substance is dissolved and in which the core material is insoluble, and then by atomizing the mixture into air stream. The air, usually heated, supplies the latent heat of vaporization required to remove the solvent from the coating material, thus forming the microencapsulated product. The equipment components of a standard spray dryer include an air heater, atomizer, main spray chamber, blower or fan, cyclone and product collector.

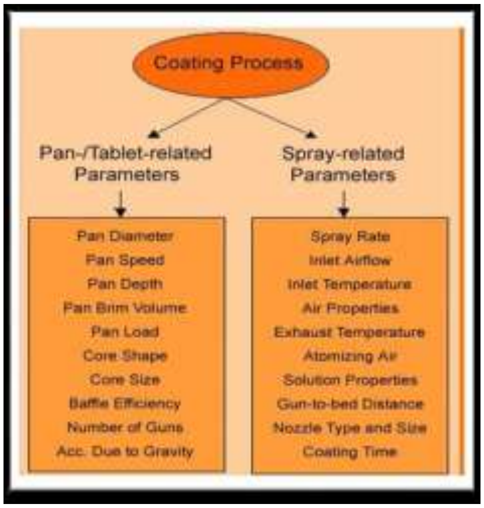

Fig.6. List of variables that affecting Spray Drying

Microencapsulation by spray congealing can be accomplished with spray drying equipment when the protective coating is applied as a melt. General process variables and conditions are quite similar to those already described, except that the core material is dispersed in a coating material melt rather than a coating solution. Coating solidification (and microencapsulation) is accomplished by spraying the hot mixture into a cool airstream. Waxes, fatty acids and alcohols, polymers and sugars, which are solids at room temperature but meltable at reasonable temperatures, are applicable to spray congealing techniques. Typically, the particle size of spray congealed products can be accurately controlled when spray drying equipment is used, and has been found to be a function of the feed rate, the atomizing wheel velocity, dispersion of feed material viscosity, and variables.

Airflow:

The initial contact between spray droplets and drying air controls evaporation rates and product temperatures in the dryer. There are three modes of contact:

1. Co-current

Drying air and particles move through the drying chamber in the same direction. Product temperatures on discharge from the dryer are lower than the exhaust air temperature, and hence this is an ideal mode for drying heat sensitive products. When operating with rotary atomizer, the air disperser creates a high degree of air rotation, giving uniform temperatures throughout the drying chamber. However, an alternative non-rotating airflow is often used in tower or FILTERMAT®-type spray dryers using nozzle atomizers with equal success.

2. Counter-current

Drying air and particles move through the drying chamber in opposite directions. This mode is suitable for products which require a degree of heat treatment during drying. The temperature of the powder leaving the dryer is usually higher than the exhaust air temperature.

3. Mixed-flow

Particle movement through the drying chamber experiences both co-current and counter-current phases. This mode is suitable for heat stable products where coarse powder requirements necessitate the use of nozzle atomizers, spraying upwards into an incoming airflow, or for heat sensitive products where the atomizer sprays droplets downwards towards an integrated fluid bed and the air inlet and outlet are located at the top of the drying chamber. eg. Study on microencapsulation of lycopene by spray drying (Bansode S.S* 2010).

f. Centrifugal extrusion

Liquids are encapsulated using a rotating extrusion head containing concentric nozzles. In this process, a jet of core liquid is surrounded by a sheath of wall solution or melt. As the jet moves through the air it breaks, owing to Rayleigh instability, into droplets of core, each coated with the wall solution. While the droplets are in flight, a molten wall may be hardened or a solvent may be evaporated from the wall solution. Since most of the droplets are within ± 10% of the mean diameter, they land in a narrow ring around the spray nozzle. Hence, if needed, the capsules can be hardened after formation by catching them in a ring-shaped hardening bath. This process is excellent for forming particles 400–2,000 µm (16–79 mils) in diameter. Since the drops are formed by the breakup of a liquid jet, the process is only suitable for liquid or slurry. A high production rate can be achieved, i.e., up to 22.5 kg (50 lb) of microcapsules can be produced per nozzle per hour per head. Heads containing 16 nozzles are available (Bansode S.S* 2010).

g. Microencapsulation processes based on ultrasonic Atomization

Ultrasonic atomization is accomplished by several means: by focusing high-frequency ultrasonic energy on the surface of a liquid in a bowl shaped transducer (0.4–10.0 MHz), by ultrasonically vibrating a surface over which the liquid flows (18–100 kHz), or by feeding the fluid into the active zone of a whistle (8–30 kHz). Small droplets of a uniform size may be formed by feeding the fluid at a controlled rate through a small orifice in the tip of a horn vibrating ultrasonically in a longitudinal mode (Fig.7). Vibration energy causes formation of liquid threads and then of droplets. Cavitation and capillary wave mechanisms are the two rival theories that have been developed. Cavitation phenomena occur at both high-energy intensity and frequency and are generated by the ultrasound wave as it passes through the liquid medium. Like any sound, the wave is transmitted as a series of compression and rarefaction cycles affecting the liquid molecules, thus generating voids (or cavities). These latter continue to grow in size until they become unstable, and then they violently collapse releasing energy to the liquid phase. The capillary wave hypothesis is based on the so-called Taylor instability, that is, the atomization occurs when unstable oscillations split the peaks of surface capillary waves (capillary waves are composed by crests or peaks and troughs) away from the liquid bulk. Since the drops are produced from peaks, their sizes are proportional to the wavelength. Among ultrasonic atomization's advantages, velocity of drops produced from an ultrasonic atomizer is 1–10% larger than that of a hydraulic or air-atomizing nozzle. Mechanical stress caused by vibration is lower, avoiding deactivation of bioactive substances. Moreover, ultrasonic atomizer operates at low energy levels (Dalmoro A. 2012).

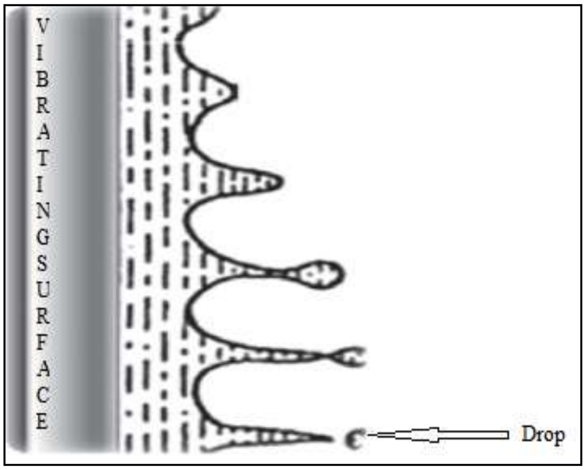

Fig no, 7 Ultrasonic atomization

2. Chemical process (Chowdhury R. S.* 2012)

a. Solvent Evaporation

This technique has been used by companies including the NCR Company, Gavaert Photo - Production NV, and Fuji Photo Film Co., Ltd. to produce microcapsules. The processes are carried out in a liquid manufacturing vehicle. The microcapsule coating is dissolved in a volatile solvent, which is immiscible with the liquid manufacturing vehicle phase. A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation, the core coating material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microcapsule. The mixture is then heated (if necessary) to evaporate the solvent for the polymer. In the case in which the core material is dispersed in the polymer solution, polymer shrinks around the core. In the case in which core material is dissolved in the coating polymer solution, a matrix - type microcapsule is formed. Once all the solvent for the

polymer is evaporated, the liquid vehicle temperature is reduced to ambient temperature (if required) with continued agitation. At this stage, the microcapsules can be used in suspension form, coated on to substrates or isolated as powders. The solvent evaporation technique to produce microcapsules is applicable to a wide variety of liquid and solid core materials. The core materials may be either water - soluble or water - insoluble materials. A variety of film - forming polymers can be used as coatings.
eg. Evaluation of Sucrose Esters as Alternative Surfactants in Microencapsulation of Proteins by the Solvent Evaporation Method.

b. Polymerization:

A. Interfacial polymer:

In Interfacial polymerization, the two reactants in a polycondensation meet at an interface and react rapidly. The basis of this method is the classical Schotten Baumann reaction between an acid chloride and a compound containing an active hydrogen atom, such as an amine or alcohol, polyesters, polyurea, polyurethane. Under the right conditions, thin flexible walls form rapidly at the interface. A solution of the pesticide and a diacid chloride are emulsified in water and an aqueous solution containing an amine and a polyfunctional isocyanate is added. Base is present to neutralize the acid formed during the reaction. Condensed polymer walls form instantaneously at the interface of the emulsion droplets.

B. In-situ polymerization:

In a few microencapsulation processes, the direct polymerization of a single monomer is carried out on the particle surface. In one process, e.g. Cellulose fibers are encapsulated in polyethylene while immersed in dry toluene. Usual deposition rates are about 0.5μm/min. Coating thickness ranges 0.2-75μm. The coating is uniform, even over sharp projections.

C. Matrix polymer:

In a number of processes, a core material is imbedded in a polymeric matrix during formation of the particles. A simple method of this type is spray-drying, in which the particle is formed by evaporation of the solvent from the matrix material. However, the solidification of the matrix also can be caused by a chemical change. Using this phenomenon, Chang prepares microcapsules containing protein solutions by incorporating the protein in the aqueous diamine phase. Chang has demonstrated the permselectivity, by their ability to convert blood urea to ammonia, the enzyme remaining within the microcapsules when incorporated within an extracorporeal shunt system. Numerous groups are utilizing polymerization techniques to accomplish microencapsulation. Examples are the National Lead Corporation, Eurand America.

Factors Influencing Encapsulation Efficiency:

The encapsulation efficiency of the microparticle or microcapsule or microsphere will be affected by different

Parameters:

I. High solubility of the polymer in organic solvent. Low solubility of organic solvent in water.
Low concentration of polymer. High DP/CP ratio.
Low solvent removal rate.
↓
Slow solidification of microparticles.
↓
Low encapsulation efficiency

II. Low solubility of the polymer in organic solvent. Solubility of organic solvent in water.
High concentration of polymer. Low DP/CP ratio.
High solvent removal rate.
↓
Fast solidification of microparticles.
↓
High encapsulation efficiency

CHARACTERIZATION:

Particle size measurement:

Different sizes of microcapsules in a batch were separated by sieve analysis using a range of standard sieves (#10, #22, #44, #52 and # 60). The amount retained on different sieves was weighed. From the obtained data, weight percent retained on different sieves and average size of microcapsules were calculated (AppaRao B* 2010).

The size of the prepared microcapsules was measured by the optical microscopy method using a calibrated stage micrometer. Particle size was calculated by using equation, Xg = 10 x [(ni x log Xi) / N], Where, Xg is geometric mean diameter, ni is number of particle in range, Xi is the mid point of range and N is the total number of particles (NAYAK B. * 2009).

Shape and surface morphology:

The Scanning Electron Microscopy (SEM) analysis was carried out using a scanning electron microscope (LEO, 435 VP, U.K.). Prior to examination, samples were mounted on an aluminium stub using a double sided adhesive tape and making it electrically conductive by coating with a thin layer of gold (approximately 20 nm) in vacuum. The scanning electron microscope was operated at an acceleration voltage of 05 kV and resolution of 4000 (NAYAK B. * 2009).

Percentage Yield:

The total amount of microcapsules obtained was weighed and the percentage yield calculated taking into consideration the weight of the drug and polymer (Shekhar K. 2010), (Sandile M. K. 2009).

$$\%\text{Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Estimation of Drug Content:

The drug content in the microcapsules was calculated by UV spectrophotometric method. The method was validated for linearity, accuracy and precision. A sample of microcapsules was dissolved in sutaiable solvent and the volume was adjusted. The solution was filtered through Whatman filter paper. Then the filtrate was assayed for drug content by measuring the absorbance after suitable dilution (Shekhar K. 2010).

%Drug content= Actual drug content of microcapsules/Theoretical weight of drug in microcapsule×100 (AppaRao B* 2010)

Encapsulation efficiency:

Encapsulation efficiency was calculated using the formula:

Encapsulation= Estimated % drug content in microcapsules/ Theoretical % drug content in microcapsules x 100 (Yadav A.V. * 2009)

Flow properties of microcapsules:

Flowability of microcapsules was investigated by determining Angle of repose, bulk density, Carr's index and Hausner ratio. The angle of repose was determined by fixed funnel method. The microcapsules were tapped using bulk density apparatus for 1000 taps in a cylinder and the changes in volume were measured. Carr index and Hausner ratio were calculated by the formula, Carr index (%) = (Df-D0) ×100 ⁄ Df and Hausner ratio = Df ⁄ D0, where, Df is poured density; D0 is tapped density.

Percentage moisture loss:

The drug loaded microcapsules was evaluated for percentage moisture loss which sharing an idea about its hydrophilic nature. The microcapsules weighed (W1) initially kept in desiccator containing calcium chloride at 37°C for 24 hours. The final weight (W2) was noted when no further change in weight of sample was observed. Moisture loss = [(W1 – W2)/ W2] X 100.

Determination of sphericity:

The particle shape was measure by computing circulatory factor (S). The tracing obtained from optical microscopy were used to calculate Area (A) and perimeter (P). This will indicate the approximate shape of the prepared microcapsule calculated by the equation, $S = P2/ 12.56 \times A$.

Loose surface crystals study:

The drug encapsulated microcapsules prepared by different combination of polymers were evaluated by loose surface crystal study to observe the excess drug present on the surface of microcapsules. From each batch, 100 mg of microcapsule was shaken in 20 ml of 0.1N HCl for 5 min and then filtered through whatman filter paper. The amount of drug present in filtrate was determined spectroscopically and calculated as a percentage of total drug content.

Determination of swelling properties

The dynamic swelling property of microcapsules in the dissolution medium was determined. Microcapsules of known weight were placed in dissolution solution for 6 hr and the swollen microcapsules were collected by a centrifuge and the wet weight of the swollen microcapsules was determined by first blotting the particles with filter paper to remove absorbed water on surface and then weighing immediately on an electronic balance. The percentage of swelling of microcapsules in the dissolution media was then calculated by using equation, $Sw = [(Wt- Wo)/Wo] \times 100$, where, Sw= percentage of swelling of microcapsules, Wt = weight of the microcapsules at time t, Wo = initial weight of the microcapsules.

Wall thickness:

Wall thickness of microcapsules was determined by the method of Luu. et. al. using equation:

$h = r (1-p) d1 / 3[pd2 + (1-p) d1]$

where as:

h = wall thickness of microcapsules

r = arithmetic mean radius

d1 = density of core material

d2 = density of coat material

p = proportion of medicament in microcapsules (NAYAK B. * 2009).

Invitro Drug release Studies:

Drug release was studied by using USP type II dissolution test apparatus (LABINDIA DISSO 2000) in Phosphate buffer of pH 7.4 (900 ml). The paddle speed at 100 rpm and bath temperature at 37 ± 0.5°c were maintained through out the experiment. A sample of microcapsules was used in each test. Aliquot equal to 5ml of dissolution medium was withdrawn at specific time interval and replaced with fresh medium to maintain sink condition. Sample was filtered through Whatman No. 1 filter paper and after suitable dilution with medium; the absorbance was determined by UV spectrophotometer (SHIMADZU 1700). All studies were conducted in triplicate (n=3) (Shekhar K. 2010) (Yadav A.V. * 2009).

KINETIC ANALYSIS OF DISSOLUTION DATA:

To study the mechanism of drug release from the microcapsules, the release data were fitted to the following equations: (Time in each case was measured in minutes)

Model 1. Zero order kinetics

$Q_1= Q_o+K_ot$

Where,

Q1-amount of drug dissolved in time t

Q0-initial amount of drug in the solution

K0-zero order release constant

Model 2. First order kinetics

$\text{In}Q_t = \text{In } Q_o+K1t$

Where,

K1--first order release constant

Q0-initial amount of drug in the solution

Q1-amount of drug dissolved in time t

Model 3.Higuchi model

$$Q = \sqrt{tDCs\ (2C-Cs)}$$

Where,

Q- Amount of drug release in time t

C- Initial drug concentration

Cs- drug solubility in the matrix

D- Diffusion constant of the drug molecule in that liquid

Model 4.Korsmeyer-Peppas:

$$\frac{M_t}{M_\infty} = at^n$$

Where,

a- constant incorporating structural and geometric characteristics of the drug dosage form

n- the release exponent (indicative of the drug release mechanism)

M_t/M_∞-fractional release of drug (Shekhar K. 2010).

APPLICATIONS OF MICROENCAPSULATION:

Some of the applications of microencapsulation can be described in detail as given below:

(a) Prolonged release dosage forms. The microencapsulated drug can be administered, as microencapsulation is perhaps most useful for the preparation of tablets, capsules or parenteral dosage forms.

(b) Microencapsulation can be used to prepare enteric coated dosage forms, so that the medicament will be selectively absorbed in the intestine rather than the stomach.

(c) It can be used to mask the taste of bitter drugs.

(d) From the mechanical point of view, microencapsulation has been used to aid in the addition of oily medicines to tabletted dosage forms. This has been used to overcome problems inherent in producing tablets from otherwise tacky granulations. This was accomplished through improved flow properties. For example, the nonflowable multicomponent solid mixture of niacin, riboflavin, and thiamine hydrochloride and iron phosphate may be encapsulated and made directly into tablets.

(e) It has been used to protect drugs from environmental hazards such as humidity, light, oxygen or heat. Microencapsulation does not yet provide a perfect barrier for materials, which degrade in the presence of oxygen, moisture or heat, however a great degree of protection against these elements can be provided. For example, vitamin A and K have been shown to be protected from moisture and oxygen through microencapsulation.

(f) The separations of incompatible substances, for example, pharmaceutical eutectics have been achieved by encapsulation. This is a case where direct contact of materials brings about liquid formation. The stability enhancement of incompatible aspirin-chlorpheniramine maleate mixture was accomplished by microencapsulating both of them before mixing.

(g) Microencapsulation can be used to decrease the volatility. An encapsulated volatile substance can be stored for longer times without substantial evaporation.

(h) Microencapsulation has also been used to decrease potential danger of handling of toxic or noxious substances. The toxicity occurred due to handling of fumigants, herbicides, insecticides and pesticides have been advantageously decreased after microencapsulation.

(i) The hygroscopic properties of many core materials may be reduced by microencapsulation.

(j) Many drugs have been microencapsulated to reduce gastric irritation.

(k) Microencapsulation method has also been proposed to prepare intrauterine contraceptive device (Chowdhury R. S.* 2012), (SACHAN N.K. * 2006).

Active moiety	**Characteristic Property**	**Purpose of encapsulation**	**Final product form**
Aspirin	Slightly soluble in water	Taste masking, sustained release, reduced in gastric irritation,	Tablet or capsule
Paracetamol	Slightly soluble in water	Taste masking	Tablet
Islet of Langerhans	Viable cells	Sustained normalization of diabetic condition	Injection
Isosorbide dinitrate	Water soluble	Sustained release	Capsule
Progesterone	Slightly soluble in water	Sustained release	Varied
Menthol	Volatile solution	Reduction in volatility, Sustained release	Lotion
Potassium chloride	Highly soluble in water	Reduction in gastric irritation	Capsule
Urease	Water soluble enzyme	Perm selectivity of enzyme, substrate and reaction	Dispersion
Vit.A Palmitate	Nonvolatile liquid	Stabilization to oxidation	Dry powder

Table 1: Examples of some microencapsulated drugs (Kumar A* 2011)**.**

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PARTICULATE DRUG DELIVERY SYSTEM: MICROSPHERE

Sawant Sandip, Pawar Yogesh

INTRODUCTION:

In contrast to drug delivery system, the word novel is searching something out of necessity. The drug has to be delivered for a prolonged period of time and many medicines have to be taken simultaneously in case of chronic patients. Frequent administration of drug is necessary when those have shorter half life and all these leads to decrease in patient's compliance. In order to overcome the above problems, various types of controlled release dosage forms are formulated and altered, so that patient compliance increase through prolonged effect , adverse effect decreases by lowering peak plasma concentration. The controlled release dosage form maintaining relatively constant drug level in the plasma by releasing the drug at a predetermined rate for an extended period of time. One such in Microspheres as carriers of drug become an approach of controlled release dosage form in novel drug delivery system. Microspheres are defined as "Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles" (or) can be defined as structure made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level (Prasanth v.v Chakraborthy Moy A. 2011). The term microcapsule is defined as a spherical particle with size varying from 50nm to 2nm containing a core substance. Alternate terminology for the microsphere is micro beads and -beads are used alternatively. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature

History: between 1940s and 1960s, the concept of chemical microencapsulation technology began as an alternative means of delivering drugs. In continued quest for the more refined systems' in 1980s polymer/Membrane technology came to be known at forefront (Tadwee I.A.K. 2012).

The dug should be delivered to specific target sites at a rate and concentration that permit optimal therapeutic efficacy while reducing side effect to minimum and patient compliance during therapy. Site Specific delivery with absolute accuracy can be achieved by attaching bioactive molecule to liposome, Bioerodable polymer, implants monoclonal antibodies and various particulate carriers (Ex. Nanoparticles and microspheres etc) the micro particulate delivery system if modified then maintains the desired concentration at the site of interest without side effect (Tadwee I.A.K. 2012) (Edlund U. 2011).

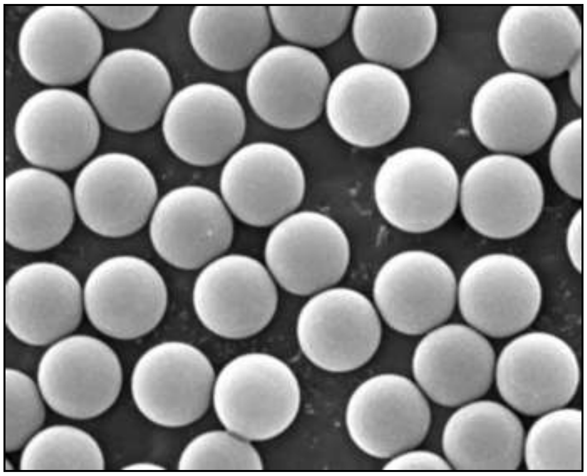

Fig.1. Microspheres

MATERIALS USED:

Types of polymer used in preparation of microspheres. Microspheres used usually are polymers. They are classified into two types.

Classification:
1. Synthetic Polymers
2. Natural polymers

1. Synthetic polymers are divided into two types.
a. Non-biodegradable polymers
E.g. Poly methyl methacrylate (PMMA)
    Acrolein
    Glycidyl methacrylate
    Epoxy polymers
b. Biodegradable polymers
E.g. Lactides, Glycolides & their co polymers,
    Poly alkyl cyano acrylates, Poly anhydrides.
2. Natural polymers obtained from different sources like proteins, carbohydrates and chemically modified carbohydrates.

Proteins: Albumin, Gelatin, and Collagen.
Carbohydrates: Agarose, Carrageenan, Chitosan, Starch.
Chemically modified carbohydrates: Poly dextran, Poly starch.

In case of non-biodegradable drug carriers, when administered parenterally, the carrier remaining in the body after the drug is completely released poses possibility of carrier toxicity over a long period of time. Biodegradable carriers which degrade in the body to non-toxic degradation products do not pose the problem of carrier toxicity and are more suited for parenteral applications (Alagusudaram M. 2009).

PREREQUISITES FOR IDEAL MICROPARTICULATE CARRIERS:

The polymers and other material utilized for the preparation of microsphere should give following characteristics: Target ability, Polyvalent, Biocompatibility, Longer duration of action, Sterilizability, Relative stability, Water solubility, Control of content release, Bioresobability, Water solubility and dispensability, Reduction of toxicity, Protection of drug (Tadwee I.A.K. 2012)**.**

METHODS OF PREPARATION:

The microspheres can be prepared by using any of the several techniques discussed in the following sections, but the choice of the technique mainly depends on the nature of the polymer used, the drug, the intended use and duration of therapy. Preparation of microspheres should satisfy certain criteria:

1. The ability to incorporate reasonably high Concentration of the drug.
2. Stability of the preparation after synthesis with a Clinically acceptable shelf life.
3. Controlled particle size and dispersibility in aqueous vehicles for injection.
4. Release of active reagent with a good control over a wide time scale.
5. Biocompatibility with a controllable biodegradability and
6. Susceptibility to chemical modification
7. There should be no toxic products associated with the final product.

1. Single emulsion technique:

The micro particulate carriers of natural polymers of natural polymers i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in non-aqueous medium like oil. Next cross linking of the dispersed globule is carried out. The cross linking can be achieved either by means of heat or by using the chemical cross linkers. The chemical cross linking agents used are glutaraldehyde, formaldehyde, diced chloride etc. Heat denaturation is not suitable for thermolabile substances. Chemical cross linking suffers the disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation and then subjected to centrifugation, washing, separation.

2. Double emulsion technique:

Double emulsion method of microspheres preparation involves the formation of the multiple emulsions or the double emulsion of type w/o/w and is best suited to water soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The continuous phase is generally consisted of the polymer solution that eventually encapsulates of the protein contained in dispersed aqueous phase. The primary emulsion is subjected then to the homogenization or the sonication before addition to the aqueous solution of the poly vinyl alcohol (PVA). This results in the formation of a double emulsion. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction. a number of hydrophilic drugs like luteinizing hormone releasing hormone (LH-RH) agonist, vaccines, proteins/peptides and conventional molecules are successfully incorporated into the microspheres using the method of double emulsion solvent evaporation/ extraction (Alagusudaram M. 2009).

Tolmetin sodium microsphere prepared by using ethylcellulose as a polymer. Microspheres were prepared by using water-in-oil-in-oil ($W/O_1/O_2$) double-emulsion solvent diffusion method, using different ratios of ethylcellulose to tolmetin sodium. Span 80 was used as the droplet stabilizer and *n*-hexane was added to harden the microspheres. The prepared microspheres were characterized for their micromeritic properties, drug content, loading efficiency, production yield, and particle size.

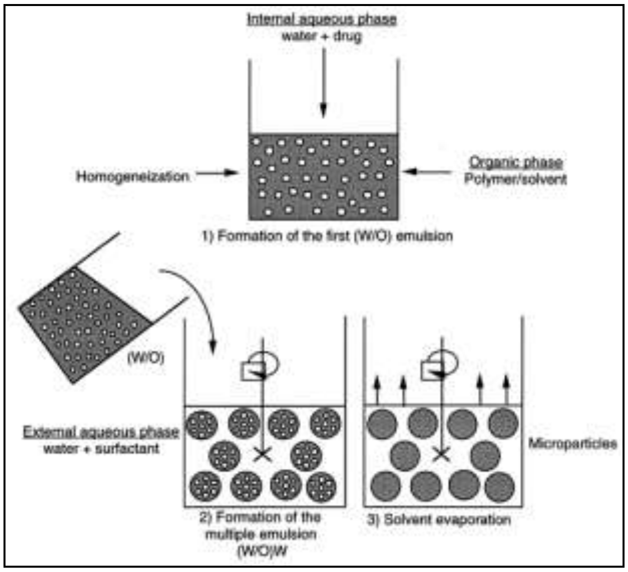

Fig.2 emulsion technique

3. Polymerization techniques:

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

I. Normal polymerization

II. Interfacial polymerization.

Both are carried out in liquid phase.

Normal polymerization:

It is carried out using different techniques as bulk, suspension, precipitation, emulsion and micellar polymerization processes. In bulk, a monomer or a mixture of monomers along with the initiator or catalyst is usually heated to initiate polymerization. Polymer so obtained may be molded as microspheres. Drug loading may be done during the process of polymerization. Suspension polymerization also referred as bead or pearl polymerization. Here it is carried out by heating the monomer or mixture of monomers as droplets dispersion in a continuous aqueous phase. The droplets may also contain an initiator and other additives. Emulsion polymerization differs from suspension polymerization as due to the presence initiator in the aqueous phase, which later on diffuses to the surface of micelles. Bulk polymerization has an advantage of formation of pure polymers.

Interfacial polymerization:

It involves the reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase.

4. Phase separation coacervation technique:

This process is based on the principle of decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates. In this method, the drug particles are dispersed in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Addition of non-solvent results in the solidification of polymer. Poly lactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer. The process variables are very important since the rate of achieving the coacervates determines the distribution of the polymer film, the particle size and agglomeration of the formed particles. The agglomeration must be avoided by stirring the suspension using a suitable speed stirrer since as the process of microspheres formation begins the formed polymerize globules start to stick and form the agglomerates. Therefore the process variables are critical as they control the kinetic of the formed particles since there is no defined state of equilibrium attainment (Alagusudaram M. 2009).

Indomethacin microspheres were prepared by phase separation co-acervation method using petroleum ether and coconut oil as dispersion and continuous phase system (Gangadhar B.C. 2010).

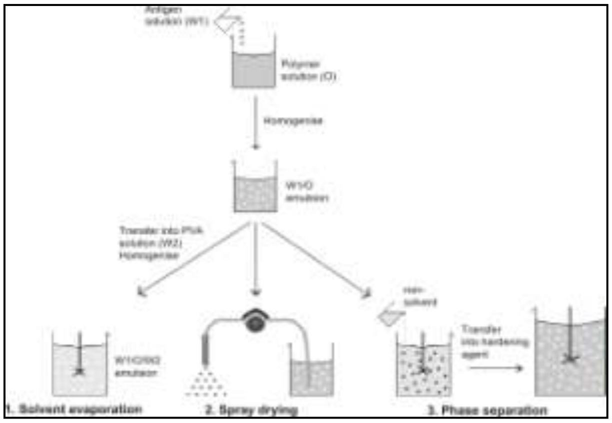

Fig.3 Phase separation

5. Spray drying and spray congealing:

These methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or cooling of the solution, the two processes are named spray drying and spray congealing respectively. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the microspheres in a size range 1-100 μm. Micro particles are separated from the hot air by means of the cyclone separator while the traces of solvent are removed by vacuum drying. One of the major advantages of the process is feasibility of operation under aseptic conditions. The spray drying process is used to encapsulate various penicillins. Thiamine mononitrate and sulpha ethylthiadizole are encapsulated in a mixture of mono- and diglycerides of stearic acid and palmitic acid using spray congealing. Very rapid solvent evaporation, however leads to the formation of porous micro particles.

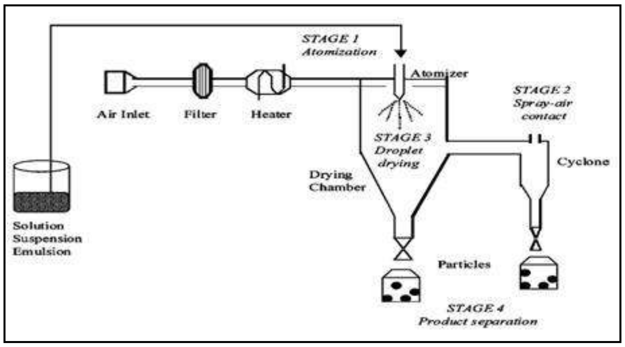

Fig.4 Spray drying

6. Solvent extraction:

Solvent evaporation method is used for the preparation of micro particles, involves removal of the organic phase by extraction of the organic solvent. The method involves water miscible organic solvents such as isopropanol. Organic phase is removed by extraction with water. This process decreases the hardening time for the microspheres. One variation of the process involves direct addition of the drug or protein to polymer organic solution. The rate of solvent removal by extraction method depends on the temperature of water, ratio of emulsion volume to the water and the solubility profile of the polymer (Alagusudaram M. 2009).

Isosorbid dinitrate microsphere where prepared by non-aqueous solvent evaporation method. The polymer used is as Acrycoat S 100 in different proportion (drug : Acrycoat S100) where dissolved in acetone. This clear solution was poured slowly as a thin stream in about 200ml of liquid paraffin solution in a 500ml beaker and stirred at different agitation speed for 1 hour, the microspheres were filtered and dried over night at 30-40^{0}c(Bhagwat D.A. 2009).

Sustained release microcapsules of acetazolamide were prepared by solvent evaporation techniques using Eudragit RL/RS as polymer (Kannan K. 2009).eg.

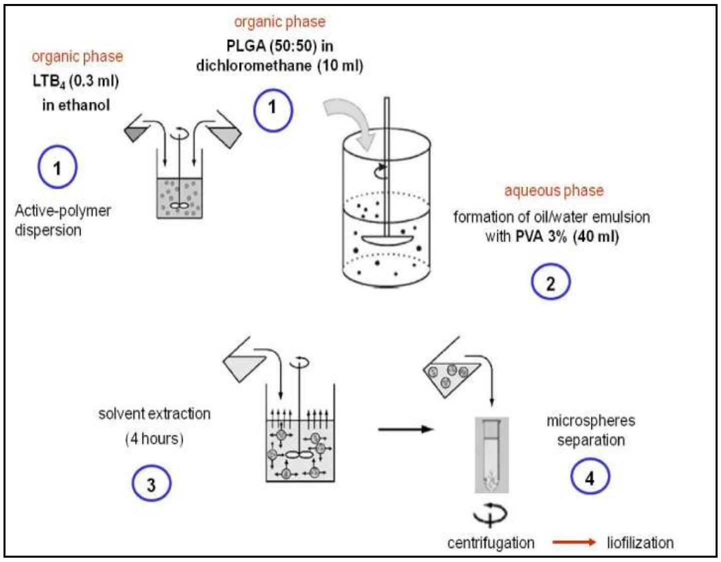
organic phase
PLGA (50:50) in dichloromethane (10 ml)
organic phase
LTB4 (0.3 ml) in ethanol
1
1
Active-polymer dispersion
aqueous phase
formation of oil/water emulsion with PVA 3% (40 ml)
2
solvent extraction (4 hours)
3
microspheres separation
4
centrifugation
liofilization

Fig.5 Solvent evaporation

7. Ionic gelation:-

Alginate/chitosan particulate system for diclofenac sodium release was prepared using this technique. 25 % (w/v) of diclofenac sodium was added to 1.2 % (w/v) aqueous solution of sodium alginate. In order to get the complete solution stirring is continued and after that it was added drop wise to a solution containing Ca2+ /Al3+ and chitosan solution in acetic acid. Microspheres which were formed were kept in original solution for 24 hr for internal jellification followed by filtration for separation. The complete release was obtained at pH 6.4-7.2 but the drug did not release in acidic pH.

8. Hydroxyl appetite (HAP) microspheres in sphere morphology:-

This was used to prepare microspheres with peculiar spheres in sphere morphology microspheres were prepared by o/w emulsion followed by solvent evaporation. At first o/w emulsion was prepared by dispersing the organic phase (Diclofenac sodium containing 5% w/w of EVA and appropriate amount of HAP) in aqueous phase of surfactant. The organic phase was dispersed in the form of tiny droplets which were surrounded by surfactant molecules this prevented the droplets from co solvencing and helped them to stay individual droplets. While stirring the DCM was slowly evaporated and the droplets solidify individual to become microspheres (Prasanth v.v 2011).

Entrapment of Drug in Microspheres:

The active component can be loaded by means of the physical entrapment, chemical linkage and surface absorption the entrapment largely depends on the method of preparation and nature of the drug or polymer. The loading is carried out in pre-formed microspheres by incubating them with high concentration of the drug in a suitable solvent (Tadwee I.A.K. 2012).

Drug Release Kinetics from Microspheres:

Release of the active constituent is an important consideration in case of microspheres. Many theoretically possible mechanisms may be considered for the release of drug from the micro particulates.

1. Liberation due to polymer erosion or degradation,
2. Self diffusion through the pore,
3. Release from the surface of the polymer,
4. Pulsed delivery initiated by the application of an oscillating or sonic field.

The release profile from the microspheres depends on the nature of the polymer used in the preparation as well as on the nature of the active drug. The release of drug from both biodegradable as well as non biodegradable microsphere(s) is influenced by structure or micro-morphology of the carrier and the properties of the polymer itself. The drugs could be released through the microspheres by any of the three methods, first is the osmotically driven burst mechanism, second pore diffusion mechanism and third by erosion or the degradation of the polymer. In osmotically driven burst mechanism, water diffuses into the core through biodegradable or non biodegradable coating, creating sufficient pressure that ruptures the membrane. The burst effect is mainly controlled by three factors the macromolecule/ polymer ratio, particle size of the dispersed macromolecule and the particle size of the microspheres. The pore diffusion method is named so because as penetrating waterfront continue to diffuse towards the core. The dispersed protein/drug dissolves creating a water filled pore network through which the active principle diffuses out in a controlled manner. In case of the biodegradable polymers, the release is controlled by both the erosion as well as diffusion process. The polymer erosion, i.e. loss of polymer is accompanied by accumulation of the Monomer in the release medium. The erosion of the polymer begins with the changes in the microstructure of the carrier as water penetrates within it leading to the plasticization of the matrix. This plasticization of the matrix finally leads to the cleavage of the hydrolytic bonds. The cleavage of the bond is also facilitated by the presence of the enzyme (lysozymes) in the surroundings. The erosion of the polymer may be either surfacial or it may be bulk leading to the rapid release of the drug active components. The rate and extent of water uptake therefore determines release profile of the system and depends on type of the polymer, porosity of the polymer matrix, protein ding loading, etc.

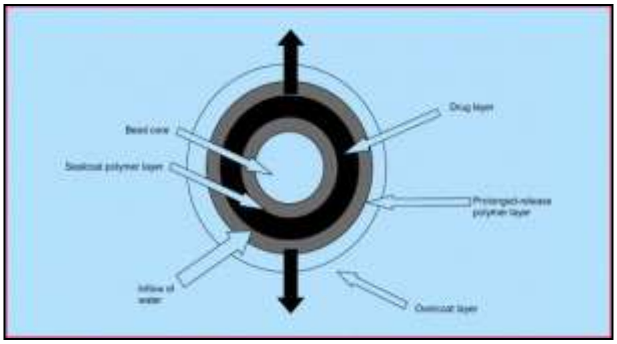

Fig.6 Cross-section of the microsphere controlled-release delivery system.

Factors affecting the release:

Controlled release is an attainable and desirable characteristic for drug delivery systems. The factors affecting the drug release rate revolve around the structure of the matrix where the drug is contained and the chemical properties associated with both the polymer and the drug. Conventional oral delivery is not rate controlled. A drug encapsulated in a slowly degrading matrix provides the opportunity for slower release effects, but polymer degradation is not the only mechanism for the release of a drug. The drug release is also diffusion controlled as the drug can travel through the pores formed during sphere hardening. In some cases, drugs containing nucleophilic groups can cause increased chain scission of the polymer matrix, which also increases the rate of drug expulsion. Polymer molecular weight, drug distribution, polymer blending, crystallinity, and other factors are important in manipulating release profiles.

Factors affecting the release from the particulate system :

- Drug:- Position in microspheres Molecular weight physicochemical properties Concentration Interaction with matrix
- Microspheres:- Type and amount of the matrix polymer Size and density of the microspheres Extent of cross linking, Denaturation or –polymerization Adjuvant.
- Environment:- PH, Polarity , Presence of enzyme (Tadwee I.A.K. 2012).

Release type of Microspheres:

A) Reservoir Type System: Release from the reservoir type system with rate controlling membrane proceeds by first penetration of the water through the membrane followed by dissolution of the drug in the penetrating dissolution fluid. The dissolved drug after partitioning through the membrane diffuses across the stagnant diffusion layer. The release is essentially governed by the fick's first law of diffusion.

B] Matrix System: Release profile of the drug from the matrix type of the device critically depends on the state of drug whether it is dissolved or dispersed in the polymer matrix. In the case of the drug dissolved in the polymeric matrix, amount of drug, and the nature of the polymer (whether hydrophobic or hydrophilic) affect the release profile (Tadwee I.A.K. 2012).

Types of Microspheres:

1. Albumin Microspheres:

Much of earlier use of serum albumin microspheres was limited to the diagnostic purpose as the microsphere of different size range locates themselves differentially at selected sites to facilitate the imaging. Because of selected uptake of protein carrier by tumor cells, the microspheres of albumin are being widely used for the targeted drug delivery to the tumor cells.

2. Gelatin Microspheres:

Gelatin microspheres are mostly studied because they are prone to strong opsonization Gelatin microspheres is prepared by cross linking gelatin in water in oil emulsion with glutaraldehyde. Opsonic gelatin microspheres are also prepared by similar method by dispersing gelatin in oil followed by cross linking with glutaraldehyde.

3. Starch Microspheres:

Starch is one of the most abundant biodegradable polymers that belong to carbohydrate class. It consists of the principle glucopyranose unit, which undergoes hydrolysis to yield D-glucose. Starch being a polysaccharide, consists of larger number of the free hydroxyl groups. By means of these free hydroxyl groups a large number of the active ingredients can be incorporated within as well as active on surface of microspheres. The starch microspheres when introduced into the body cavity undergo potential swelling, leading to the development of mucoadhesive character. Therefore, they are not cleared rapidly from the body cavity. Intra nasally administered insulin starch microspheres are cleared slowly and offer a delivery mode for protein and small molecules.

4. Dextran Microspheres:

Dextran, a carbohydrate is used to prepare hydrogel type of biodegradable and biocompatible systems. It can be chemically modified that provide higher percentage of drug or proteins incorporation. Simple method of incorporating aldehydes group to dextran is by oxidation using sodium iodate Protein loaded dextran microspheres are prepared by water in oil emulsion technique. In this method an aqueous solution of the methacrylated dextran is emulsified in aqueous solution of polyethylene glycol (PEG). The dispersed methacrylated phase is then cross-linked by using radical polymerization of the dextran bound methacrylate groups. This leads to the formation of the dextran microspheres with hydrogel character.

5. Poly Lactide and poly Glycolide Microspheres:

Poly (lactic acid) (PLA), poly (glycolic acid) (PGA) and their copolymer poly (lactide co glycolide) (PLGA) represent the group of synthetic biodegradable polymers. They were used earlier as absorbable sutures, implant material and recently as the carrier for the drug. L poly (lactic acid) has been reported as a suitable carrier for sustained release. narcotic antagonist and anticancer agents such as cisplatin, cyclophosphamide and doxorubicin.

6. Polyanhydride Microspheres:

Polyanhydride are biodegradable and biocompatible polymers. They were first prepared using aromatic monomers. The Polyanhydride can be manufactured with desired features such as crystallinity, controlled degradation rate, degree cross-linking, water uptake, etc. Polyanhydride microspheres can be prepared % solvent evaporation, solvent extraction, hot mete technique and spray drying techniques. For the bet melt encapsulation procedure.

7. Chitosan microsphere:

The effect of Chitosan has been considered mainly because of its positive charge; however, the adsorption process could also be the result of other forces might exist between molecules, such as hydrogen bonding or Vander Waal's forces. These interactions might have a strong impact on the absorption and bioavailability of pharmaceutical Due to attractive properties and wider applications of Chitosan-based microcapsules and microspheres they are used as a carrier for the applications in controlled drug release.

8. Polyphosphazene Microspheres:

Polyphosphazene polyacids are potent immune stimulating compounds Polyphosphazene polymers have a long chain backbone of alternating nitrogen and phosphorus atom with two side group attached to each phosphorus atom Polyphosphazene polymers form highly swollen ionotropic gel in the presence of the multivalent ions in aqueous media, such as calcium. Because of this property the polyphosphazene microspheres can be prepared under very mild conditions of low temperature and in absence of the organic solvent the microspheres are prepared by using a droplet apparatus, which produces spherical gel particle of size range 0.5-1.5 um. In this method a 2.5% w/v phosphazene solution is added in the form of the droplets to a 7.5% w/v aqueous solution of calcium chloride.

9. Other Important types of microspheres:

Polysaccharides or Lipid Cross linked Chitosan Microspheres, Alginate microsphere, Carrageenan polysaccharide or lipid cross linked chitosan microsphere, Poly alkyl cyanoacrylate microsphere, and Poly Acrolein microspheres (Tadwee I.A.K. 2012).

FATE OF MICROSPHERES IN BODY:

Microparticulate carrier system can be administered through different routes such as intravenous, ocular, intramuscular, intra arterial, oral,etc .The micro particles are intended to be administered through different routes to achieve desired activity of either sustained action or targeting or both. Through different routes different mechanism of uptake, transport and fate of translocated particles have been proposed. The polystyrene microspheres administered orally are reported to be taken up by Peyer’s patch. They are subsequently translocated to discrete anatomical compartments such as mesenteric lymph vessels, lymph nodes and to lesser extent in liver and spleen (Sanders and Ashworth 1961,Jani et al.,1990).The particulate matters gain entry into follicle associated epithelium through Peyer’s patches.

After the uptake of the particulate carrier via different mechanism their fate becomes important. Some uptake mechanisms avoid the lysosomal system of the enterocytes. The particles following uptake by enterocytes are transported to the mesenteric lymph, followed by systemic circulation and are subsequently phagocytosized by the kupffer cells of liver. However, after uptake by enterocytes, some particulate carriers may be taken up in to vacuoles and discharged back into gut lumen (Alpar et al.,1989).

Microspheres can also be designed for the controlled release to the gastrointestinal tract. The release of the drug content within microspheres. The release of the drug could be regulated by selecting an appropriate hydrophilic/lipophilic balance of the matrix such as in case of matrix of polyglycerol esters of fatty acid (Ebel,1990). Micro particles of mucoadhesive polymers get attached to the mucous layer in GIT and hence prolong the gastric residence time and functionally offer a sustained drug release. The microspheres of particle size less than 0.87micrometer are taken to the general circulation. The fluid environment of the GIT can affect the number and rate of particles translocation.

Microsphere given by parenteral route (intravenous) distribute themselves according to their size rang. After intravenous administration the particulate carriers distribute themselves passively or if suitably designed, then actively. This distribution is referred to as passive mode of the site specific delivery of the micro particulates (V. S. R.K. 2007).

Evaluation And Characterization Of Microsphere:

The characterization of the microparticulate carrier is an important phenomenon, which helps to design a suitable carrier for the proteins, drug or antigen delivery. These microspheres have different microstructures. These microstructures determine the release and the stability of the carrier.

1. Particle size and shape :

The most widely used procedures to visualize micro particles are conventional light microscopy (LM) and scanning electron microscopy (SEM). Both can be used to determine the shape and outer structure of micro particles. LM provides a control over coating parameters in case of double walled microspheres. The microspheres structures can be visualized before and after coating and the change can be measured microscopically. SEM provides higher resolution in contrast to the LM. SEM allows investigations of the microspheres surfaces and after particles are cross-sectioned, it can also be used for the investigation of double walled systems. Conflocal fluorescence microscopy is used for the structure characterization of multiple walled microspheres. Laser light scattering and multi size coulter counter other than instrumental methods, which can be used for the characterization of size, shape and morphology of the microspheres (Daiane F. dos Santos a 2011).

2. Electron spectroscopy for chemical analysis:-

The surface chemistry of the microspheres can be determined using the electron spectroscopy for chemical analysis (ESCA). ESCA provides a means for the determination of the atomic composition of the surface. The spectra obtained using ESCA can be used to determine the surfacial degradation of the biodegradable microspheres.

3. Attenuated total reflectance Fourier Transfom Infrared Spectroscopy:-

FT-IR is used to determine the degradation of the polymeric matrix of the carrier system. The surface of the microspheres is investigated measuring alternated total reflectance (ATR). The IR beam passing through the ATR cell reflected many times through the sample to provide IR spectra mainly of surface material. The ATRFTIR provides information about the surface composition of the microspheres depending upon manufacturing procedures and conditions.

4. Density determination:

The density of the microspheres can be measured by using a multi volume pycnometer. Accurately weighed sample in a cup is placed into the multi volume pycnometer. Helium is introduced at a constant pressure in the chamber and allowed to expand. This expansion results in a decrease in pressure within the chamber. Two consecutive readings of reduction in pressure at different initial pressure are noted. From two pressure readings the volume and hence the density of the microsphere carrier is determined.

5. Isoelectric point:

The micro electrophoresis is an apparatus used to measure the electrophoretic mobility of microspheres from which the isoelectric point can be determined. The mean velocity at different Ph values ranging from 3-10 is calculated by measuring the time of particle movement over a distance of 1 mm. By using this data the electrical mobility of the particle can be determined. The electrophoretic mobility can be related to surface contained charge, ionisable behaviour or ion absorption nature of the microspheres.

6. Surface carboxylic acid residue:

The surface carboxylic acid residue is measured by using radioactive glycine. The radioactive glycine conjugates is prepared by the reaction of c14-glycine ethyl ester hydro chloride with the microspheres. The glycine residue is linked using the water soluble condensing 1- ethyl-3 (3-dimethyl amino propyl) carbidiimide (EDAC). The radioactivity of the conjugate is then measured using liquid scintillation counter. Thus the carboxylic acid residue can be compared and correlated. The free carboxylic acid residue can be measured for hydrophobic or hydrophilic or any other derivatized type of the microspheres.

7. Surface amino acid residue:

Surface associated amino acid residue is determined by the radioactive c14-acetic acid conjugate. The carboxylic acid residue is measured through the liquid scintillation counter and hence the amino acid residue can be determined indirectly. EDAC is used to condense the amino group and the c14 –acetic acid carboxylic acid residue. The method used for determining the free amino or the free carboxylic acid residues are based on indirect estimation, by measuring the radioactivity of the c14 having acetic acid or the glycine conjugate. The accuracy of the method however, depends on the time allowed for conjugation of the radioactive moiety and the reactivity of free functional group.

8. Capture efficiency:

The capture efficiency of the microspheres or the percent entrapment can be determined by allowing washed microspheres to lyse. The lysate is then subjected to the determination of active constituents as per monograph requirement. The percent encapsulation efficiency is calculated using following equation:

% Entrapment = Actual content/Theoretical content x 100

9. Angle of contact:

The angle of contact is measured to determine the wetting property of a micro particulate carrier. It determines the nature of microspheres in terms of hydrophilicity or hydrophobicity. This thermodynamic property is specific to solid and affected by the presence of the adsorbed component. The angle of contact is measured at the solid/air/water interface. The advancing and receding angle of contact are measured by placing a droplet in a circular cell mounted above objective of inverted microscope. Contact angle is measured at 200C within a minute of deposition of microspheres.

10. In vitro methods:

There is a need for experimental methods which allow the release characteristics and permeability of a drug through membrane to be determined. For this purpose, a number of *in vitro* and *in vivo* techniques have been reported. *In vitro* drug release studies have been employed as a quality control procedure in pharmaceutical production, in product development etc. Sensitive and reproducible release data derived from physico chemically and hydro dynamically defined conditions are necessary. The influence of technologically defined conditions and difficulty in simulating *in vivo* conditions has led to development of a number of *in vitro* release methods for buccal formulations; however no standard *in vitro* method has yet been developed. Different workers have used apparatus of varying designs and under varying conditions, depending on the shape and application of the dosage form developed.

11. Beaker method:

The dosage form in this method is made to adhere at the bottom of the beaker containing the medium and stirred uniformly using over head stirrer. Volume of the medium used in the literature for the studies varies from 50-500 ml and the stirrer speed form 60-300 rpm.

12. Interface diffusion system:

This method is developed by Dearden & Tomlinson. It consists of four compartments. The compartment A represents the oral cavity, and initially contained an appropriate concentration of drug in a buffer. The compartment B representing the buccal membrane, contained 1-octanol, and compartment C representing body fluids, contained 0.2 M HCl. The compartment D representing protein binding also contained 1-octanol. Before use, the aqueous phase and 1-octanol were saturated with each other. Samples were withdrawn and returned to compartment A with a syringe.

13. Modified Keshary Chien Cell:

A specialized apparatus was designed in the laboratory. It comprised of a Keshary Chien cell containing distilled water (50ml) at 370 C as dissolution medium. TMDDS (Trans Membrane Drug Delivery System) was placed in a glass tube fitted with a 10# sieve at the bottom which reciprocated in the medium at 30 strokes per min.

14. Dissolution apparatus:

Standard USP or BP dissolution apparatus have been used to study *in vitro* release profiles using rotating elements, paddle and basket. Dissolution medium used for the study varied from 100-500 ml and speed of rotation from 50-100 rpm.

15. Other methods:

Few other methods involving plexi glass sample blocks placed in flasks30, agar gel method, Valia-Chein cell USP n2 III dissolution apparatus, etc have also been reported. Although a number of methods have been reported, the ideal method would be one where sink condition is maintained and dissolution time *in vitro* simulates dissolution time *in vivo.*

a. In vivo methods:

Methods for studying the permeability of intact mucosa comprise of techniques that exploit the biological response of the organism locally or systemically and those that involve direct local measurement of uptake or accumulation of penetrants at the surface. Some of the earliest and simple studies of mucosal permeability utilized the systemic pharmacological effects produced by drugs after application to the oral mucosa. However the most widely used methods include *in vivo* studies using animal models, buccal absorption tests, and perfusion chambers for studying drug permeability.

b. Animal models:

Animal models are used mainly for the screening of the series of compounds, investigating the mechanisms and usefulness of permeation enhancers or evaluating a set of formulations. A number of animal models have been reported in the literature, however, very few *in vivo* (animal). Animal models such as the dog, rats, rabbits, cat, hamster, pigs, and sheep have been reported. In general, the procedure involves anesthetizing the animal followed by administration of the dosage form. In case of rats, the oesophagus is ligated to prevent absorption pathways other than oral mucosa. At different time intervals, the blood is withdrawn and analyzed.

c. Buccal absorption test:

The buccal absorption test was developed by Beckett & Triggs in 1967. It is a simple and reliable method for measuring the extent of drug loss of the human oral cavity for single and multi component mixtures of drugs. The test has been successfully used to investigate the relative importance of drug structure, contact time, initial drug concentration and Ph of the solution while the drug is held in the oral cavity45.

d. In vitro-In vivo correlations:

Correlations between in vitro dissolution rates and the rate and extent of availability as determined by blood concentration and or urinary excretion of drug or metabolites are referred to as "*in vitro-in vivo* correlations". Such correlations allow one to develop product specifications with bioavailability.

e. Percent of Drug Dissolved *In Vitro* Vs Peak Plasma Concentration:

One of the ways of checking the in vitro and in vivo correlation is to measure the percent of the drug released from different dosage forms and also to estimate the peak plasma concentrations achieved by them and then to check the correlation between them. It is expected that a poorly formulated dosage form releases amount of drug than a well formulated dosage form, and, hence the amount of drug available for absorption is less for poorly formulated dosage form than from a well formulated dosage form.

f. Percent of Drug Dissolved Vs Percent of Drug Absorbed:

If the dissolution rate is the limiting step in the absorption of the drug, and is absorbed completely after dissolution, a linear correlation may be obtained by comparing the percent of the drug absorbed to the percent of the drug dissolved. If the rate limiting step in the bioavailability of the drug is the rate of absorption of the drug, a change in the dissolution rate may not be reflected in a change in the rate and the extent of drug absorption from the dosage form.

g. Dissolution Rate Vs Absorption Rate:

The absorption rate is usually more difficult to determine than the absorption time. Since the absorption rate and absorption time of a drug are inversely correlated, the absorption time may be used in correlating the dissolution data to the absorption data. In the analysis of in vitro and in vivo drug correlation, rapid drug absorption may be distinguished from the slower drug absorption by observation of the absorption time for the dosage form. The quicker the absorption of the drug the less is the absorption time required for the absorption of the certain amount of the drug. The time required for the absorption of the same amount of drug from the dosage form is correlated.

g. Percent of Drug Dissolved Vs Serum Drug Concentration:

For drugs whose absorption from GIT is dissolution rate limited, a linear correlation may be established between the percent of drug dissolved at specified times and the serum drug concentrations at corresponding times.

h. Percent of Drug Dissolved Vs Percent of the Dose Excreted in urine:

The percent of a drug dissolved and the percent of drug absorbed are linearly correlated. There exists a correlation between the amount of drug in body and the amount of drug excreted in the urine. Therefore, a linear relation may be established between the percent of the drug dissolved and the percent of the dose excreted in the urine (Alagusudaram M. 2009).

APPLICATIONS:

1. Microspheres in Vaccine Delivery:-Microsphere played vital role in vaccine delivery for protection against the microorganism or their toxic product Biodegradable delivery system for vaccines is given by parenteral route. The interest in parenteral carrier lies since they offer specific advantages including improved antigenicity by adjuvant action, Modulation of antigen release and Stabilization of antigen.

2. Magnetic Microspheres:- Magnetic monitoring has the advantage of being efficient in allowing high local concentration of therapeutic agents. A variety of magnetically responsive carriers have been proposed for chemo-therapeutic agents. These include magnetite containing matrices (microspheres or nanoparticles of the starch, albumin, ethyl cellulose, etc.), ethyloleate based emulsion and natural cells such as erythrocyte ghosts. Magnetic targeting is one of the most efficient methods developed for targeting of active agents. Magnetic microspheres are prepared by mixing water soluble drugs and 10 nm magnetite Fe_3O_4 particles in an aqueous solvent of matrix material. This mixture is then emulsified in the oil. Ultrasonication or shearing is done to produce particle of suitable size range. Magnetic microspheres are administered via intra-arterial or intravenous injection. Intra-arterial injection is given to achieve high systemic targeting while intravenous administration helps in achieving high pulmonary targeting.

3. Monoclonal Antibodies Mediated Microspheres Targeting- (Immunomicrospheres):

Monoclonal antibodies mediated targeting is a method used to achieve selective targeting to the specific sites. Monoclonal antibodies are extremely specific molecules. And can be utilized to target microspheres loaded bioactive molecules to selected sites. The Monoclonal Antibodies can be attached to the microspheres by any of the following methods:

1. Non specific adsorption
2. Specific adsorption
3. Direct coupling
4. Coupling via reagents

4. Chemoembolization:

Chemoembolization is an endovascular therapy, which involves the selective arterial Embolization of a tumor together with simultaneous or subsequent local delivery of the chemotherapeutic agent. Chemoembolization is an extension of traditional percutaneous Embolization techniques. With Chemoembolization, investigators embolize tumors with micro particles soaked with chemotherapeutic agents. The theoretical advantage is that such embolizations will not only provide vascular occlusion but will bring about sustained therapeutic levels of chemotherapeutics in the areas of tumor.

5. Topical porous microspheres (Microsponges): Microsponges are porous microspheres posses inter connected voids of particle size range 5-300 um. These microsponges having capacity to entrap wide range of active ingredients such as emollients, fragrances, essential oils, sunscreens and anti-infectives, etc these porous microspheres with active ingredients can be incorporated in to formulations such as creams, lotions and powders. Microsponges consist of non collapsible structures with porous surface through which active ingredients are released in a controlled manner. The Micro sponge system can reduce significantly the irritation of effective drugs without reducing their efficacy.

6. Route targeting:

Oral:

The controlled release systems have been developed for oral administration14. Oral route is also suggested for the delivery of the soluble antigens this as viable alternative can be considered due to the ability of the particles of definite size range. The risk of dose dumping is minimized with this formulation the smaller size of particles and high drug loaded particles show faster release and oral route is also suggested for the delivery of the soluble antigens.

Intranasal:

In this type of targeting the microspheres is given at the surface of nasal mucosa by considering the muco ciliary clearance. The particle size range of microspheres for targeting the respiratory tract is given table**.**

Ocular:

The eye and the cornea are easily accessible targets. The washout effect, however, presents difficulties in retention of micro particulate drug carrier in the corneal sac. The rapid conversion of the particulate suspension to gel form reportedly leads to their longer retention in the eye (Tadwee I.A.K. 2012).\

Table no.1- particle size of the microspheres for targeting of specific area is as follows:

Respiratory part	**Required Particle size (um)**
Nose	25-30
Throat	20-28
Pharynx	20-24
Larynx	15-20
Trachea	10-15
Bronchi	8-12
Bronchioles	8-10
Alveolar duct	5-8
Alveoli	4-8

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PARTICULATE DRUG DELIVERY SYSTEM- RESEALED ERYTHROCYTES

Shinde Rohit, Shaikh Aamir

INTRODUCTION:

Resealed erythrocytes part of parental control release formulation consisting erythrocyte, also known as red blood cells, have been extensively studied for their potential carrier capabilities for the delivery of drug and drug loaded microspheres. (Ilher 1989) Such drug loaded carrier erythrocyte are prepared simply by collecting blood samples from the organism of interest ,separating erythrocyte from plasma ,entrapping the drug in the erythrocyte and resealing the resultant cellular carriers. Hence these carriers are called resealed erythrocyte. The overall process is based on the response of these cells under osmotic conditions. Upon reinjection, the drug-loaded erythrocytes serve as slow circulating depots and target the drugs to disease tissue or organ . (rajendra 2011)

ERYTHROCYTES:

Erythrocytes are natural products of the body, biodegradable in nature, isolation of these is easy and large amount of drug can be loaded in small volume of cells, non immunogenic in action and can be targeted to disease tissue or organ, prolong the systemic activity of the drug while residing for a longer time in the body, protect the premature degradation, inactivation and excretion, of proteins and enzymes, act as a carrier for number of drugs, target the drugs within the reticuloendothelial system (RES) as well non RES organs/sites. They have the capacity to carry large amounts of drug; and can behave as a slow-release longacting system. Potential clinical indications for “RES targeting” include iron over-storage diseases, parasitic diseases, hepatic tumors, cancer and lysosomal storage diseases carriers. (rajendra 2011)

ERYTHROCYTES CAN BE USED AS CARRIERS IN TWO WAYS:

1. Targeting particular tissue/organ:

For targeting, only the erythrocyte membrane is used. This is obtained by splitting the cell in hypotonic solution and after introducing the drug into the cells, allowing them to reseal into spheres. Such erythrocytes are called Red cell ghosts.

2. For continuous or prolonged release of drugs:

Alternatively, erythrocytes can be used as a continuous or prolonged release system, which provide prolonged drug action. There are different methods for encapsulation of drugs within erythrocytes. They remain in the circulation for prolonged periods of time (up to 120 days) and release the entrapped drug at a slow and steady rate. (rajendra 2011)

MORPHOLOGY AND PHYSIOLOGY OF ERYTHROCYTES:

Erythrocytes are the most abundant cells in the human body (~5.4 million cells/mm3 blood in a healthy male and ~ 4.8 million cells/mm3 blood in a healthy female). These cells were described in human blood samples by Dutch Scientist Lee Van Hock in 1674. In the 19th century, Hope Seyler identified hemoglobin and its crucial role in oxygen delivery to various parts of the body . Erythrocytes are biconcave discs with an average diameter of 7.8 µm, a thickness of 2.5µm in periphery, 1 µm in the center, and a volume of 85–9µm3 . The flexible, biconcave shape enables erythrocytes to squeeze through narrow capillaries, which may be only 3µm wide. Mature erythrocytes are quite simple in structure. They lack a nucleus and other organelles Their plasma membrane encloses hemoglobin, a heme-containing protein that is responsible for O2–CO2 binding inside the erythrocytes. The main role of erythrocytes is the transport of O2 from the lungs to tissues and the CO2 produced in tissues back to lungs. Thus, erythrocytes are a highly specialized O2 carrier system in the body. Because a nucleus is absent, all the intracellular space is available for O2 transport. Also, because mitochondria are absent and because energy is generated anaerobically in erythrocytes, these cells do not consume any of the oxygen they are carrying.

Erythrocytes live only about 120 days because of wear and tear on their plasma membranes as they squeeze through the narrow blood capillaries. Worn-out erythrocytes are removed from circulation and destroyed in the spleen and liver (RES), and the breakdown products are recycled. The process of erythrocyte formation within the body is known as *erythropoiesis*. In a mature human being, erythrocytes are produced in red bone marrow under the regulation of a hemopoietic hormone called *erythropoietin.* (Gothoskar 2004)

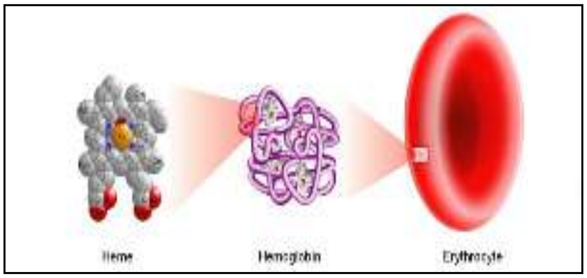

FIG 1: ***COMPOSITION OF ERYTHROCYTE***

FACTORS CONSIDERING RESEALED ERYTHROCYTES AS CARRIER:

1. Its shape and size to permit the passage through the capillaries.

2. Its specific physico-chemical properties by which a prerequisite site can be recognized.

3. Its biocompatible and minimum toxicity character.

4. Its degradation product, after release of the drug at the target site, should be biocompatible.

5. Low leaching/leakage of drug should take place before target site is reached.

6. Its drug released pattern in a controlled manner.

7. High drug loading efficiency for broad spectrum of drugs with different properties.

8. Physicochemical compatibility with the drug.

9. The carrier system should have an appreciable stability during storage. (Sha 2011)

ADVANTAGES OF ERYTHROCYTES AS DRUG CARRIERS:

Advantages include:

1. Their biocompatibility, particularly when autologous cells are used, hence no possibility of triggered immune response.
2. Their biodegradability with no generation of toxic products.
3. The considerably uniform size and shape of the carrier.
4. Relatively inert intracellular environment.
5. Prevention of degradation of the loaded drug from inactivation by endogenous chemicals.
6. The wide variety of chemicals that can be entrapped.
7. The modification of pharmacokinetic and pharmacodynamic parameters of drug.
8. Attainment of steady-state plasma concentration decreases fluctuations in concentration.
9. Protection of the organism against toxic effects of drugs (e.g. antineoplastics).

Other advantages are:

10. Their ability to circulate throughout the body.
11. The availability of the techniques and facilities for separation, handling, transfusion, and working with erythrocytes.
12. The prevention of any undesired immune response against the loaded drug.
13. Their ability to target the organs of the RES.
14. The possibility of ideal zero-order drug-release kinetics.
15. The lack of occurrence of undesired immune response against encapsulated drug.
16. The large quantity of drug that can be encapsulated within a small volume of cells ensures dose sufficiency.
17. A longer life span in circulation as compared with other synthetic carriers, and optimum conditions may result in the life span comparable to that of normal erythrocytes.

18. Easy control during life span ranging from minutes to months.
19. A decrease in side effects of drugs.
20. A considerable increase in drug dosing interval with drug residing in therapeutic window region for longer time periods. (Gothoskar 2004)

DISADVANTAGES:

1. They have a limited potential as carrier to non-phagocyte target tissue.
2. Possibility of clumping of cells and dose dumping may be there.
3. The major problem encountered in the use of biodegradable materials or natural cells as drug carriers is that they are removed in vivo by the RES as result of modification that occurred during loading procedure in cells. This, although expands the capability to drug targeting to RES, seriously limits their life-span as long-circulating drug carriers in circulation and, in some cases, may pose toxicological problems.
4. The rapid leakage of certain encapsulated substances from the loaded erythrocytes.
5. Several molecules may alter the physiology of the erythrocyte.
6. Given that they are carriers of biological origin, encapsulated erythrocytes may present some inherent variations in their loading and characteristics compared to other carrier systems.
7. The storage of the loaded erythrocytes is a further problem provided that there are viable cells and need to survive in circulation for a long time upon re-entry to the host body. Conditioning carrier cells in isotonic buffers containing all essential nutrients, as well as in low temperatures, the addition of nucleosides or chelators, lyophilization with glycerol or gel immobilization have all been exploited to overcome this problem.
8. Possible contamination due to the origin of the blood, the equipment used and the loading environment. Rigorous controls are required accordingly for the collection and handling of the erythrocytes. (Hamidi 2007)

SOURCE AND ISOLATION OF ERYTHROCYTES:

Various types of mammalian erythrocytes have been used for drug delivery, including erythrocytes of mice, cattle, pigs, dogs, sheep, goats, monkeys, chicken, rats, and rabbits. To isolate erythrocytes, blood is collected in heparinized tubes by vein puncture. (Chasis 1986) Fresh whole blood is typically used for loading purposes because the encapsulation efficiency of the erythrocytes isolated from fresh blood is higher than that of the aged blood. Fresh whole blood is the blood that is collected and immediately chilled to 4°C and stored for less than two days. The erythrocytes are then harvested and washed by centrifugation. The washed cells are suspended in buffer solutions at various hematocrit values as desired and are often stored in acid–citrate–dextrose buffer at 4°C for as long as 48 h before use. Jain and Vyas have described a well-established protocol for the isolation of erythrocytes. In 1953, Gardos tried to load erythrocyte ghost using adenosine triphosphate (ATP). In 1959,Marsden and Ostting reported the entrapment of dextran (molecular weight 10–250 kDa). In 1973, the loading of drugs in erythrocytes was reported separately by Ihler et al. and Zimmermann . In 1979, the term *carrier erythrocytes* were coined to describe drug-loaded erythrocytes. (Gothoskar 2004)

PROPERTIES OF RESEALED ERYTHROCYTE OF NOVEL DRUG DELIVERY CARRIERS:

1) The drug should be released at target site in a controlled manner

2) It should be appropriate size, shape and should permit the passage through capillaries and minimum leakage of drug should take place.

3) It should be biocompatible and should have minimum toxic effect.

4) It should possess the ability to carry a broad spectrum of drug.

5) It should possess specific physicochemical properties by which desired target size could be recognized.

6) The degradation product of the carriers system , after release of the drug at the selected site should be biocompatible. It should be physiochemically compatible with drug.

7) The carrier system should have an appreciable stability during storage. (Shah 2011)

METHODS OF DRUG LOADING:

Several methods can be used to load drugs or other bioactive compounds in erythrocytes including physical (e.g., electrical pulse method) osmosis-based systems, and chemical methods (e.g., chemical perturbation of the erythrocytes, membrane).

1. Hypotonic hemolysis:

This method is based on the ability of erythrocytes to undergo reversible swelling in a hypotonic solution. Erythrocytes have an exceptional capability for reversible shape changes with or without accompanying volume change and for reversible deformation under stress. An increase in volume leads to an initial change in the shape from biconcave to spherical. This change is attributable to the absence of superfluous membrane; hence the surface area of the cell is fixed. The cells assume a spherical shape to accommodate additional volume while keeping the surface area constant. The volume gain is ~25–50%. The cells can maintain their integrity up to a tonicity of ~150 m osm/kg, above which the membrane ruptures, releasing the cellular contents. At this point (just before cell lysis), some transient pores of 200–500 Å are generated on the membrane. After cell lysis, cellular contents are depleted. The remnant is called an erythrocyte ghost.

The principle of using these ruptured erythrocytes as drug carriers is based on the fact that the ruptured membranes can be resealed by restoring isotonic conditions. Upon incubation, the cells resume their original biconcave shape and recover original impermeability (Gothoskar 2004)

2. Use of red cell loader:

Magnani et al. developed a novel method for entrapment of nondiffusible drugs into erythrocytes. They developed a piece of equipment called a “red cell loader” .With as little as 50 mL of a blood sample, different biologically active compounds were entrapped into erythrocytes within a period of 2 h at room temperature under blood banking conditions. The process is based on two sequential hypotonic dilutions of washed erythrocytes followed by concentration with a hemofilter and an isotonic resealing of the cells. There was ~30% drug loading with 35–50% cell recovery. The processed erythrocytes had normal survival in vivo. The same cells could be used for targeting by improving their recognition by tissue macrophages. (Gothoskar 2004)

3. Hypotonic dilution:

Hypotonic dilution was the first method investigated for the encapsulation of chemicals into erythrocytes and is the simplest and fastest . In this method, a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug. The solution tonicity is then restored by adding a hypertonic buffer. The resultant mixture is then centrifuged, the supernatant is discarded, and the pellet is washed with isotonic buffer solution. The major drawbacks of this method include low entrapment efficiency and a considerable loss of hemoglobin and other cell components . This reduces the circulation half life of the loaded cells. These cells are readily phagocytosed by RES macrophages and hence can be used for targeting RES organs. Hypotonic dilution is used for loading enzymes such as β-galactosidase and β-glucosidase, asparginase, and arginase as well as bronchodilators such as salbutamol. (Gothoskar 2004)

4. Hypotonic preswelling:

This method was developed by Rechsteiner in 1975 and was modified by Jenner et al. for drug loading. The technique is based upon initial controlled swelling in a hypotonic buffered solution. This mixture is centrifuged at low *g* values. The supernatant is discarded and the cell fraction is brought to the lysis point by adding 100–120 µL portions of an aqueous solution of the drug to be encapsulated. The mixture is centrifuged between the drug-addition steps. The lysis point is detected by the disappearance of a distinct boundary between the cell fraction and the supernatant upon centrifugation. The tonicity of a cell mixture is restored at the lysis point by adding a calculated amount of hypertonic buffer. Then, the cell suspension is incubated at 37°C to reanneal the resealed erythrocytes . Such cells have a circulation half life comparable to that of normal cells . This method is simpler and faster than other methods, causing minimum damage to cells. Drugs encapsulated in erythrocytes using this method include propranolol , asparginase , cyclopohphamide, cortisol-21-phosphate ,$_{\omega}$1-antitrypsin, methotrexate, insulin , metronidazole , levothyroxine , enalaprilat , and isoniazid. (Gothoskar 2004)

5. Hypotonic dialysis:

This method was first reported by Klibansky in 1959 and was used in 1977 by Deloach and Ihler , and Dale for loading enzymes and lipids. Several methods are based on the principle that semipermeable dialysis membrane maximizes the intracellular: extracellular volume ratio for macromolecules during lysis and resealing. In the process, an isotonic, buffered suspension of erythrocytes with a hematocrit value of 70–80 is prepared and placed in a conventional dialysis tube immersed in 10–20 volumes of a hypotonic buffer. The medium is agitated slowly for 2 h. The tonicity of the dialysis tube is restored by directly adding a calculated amount of a hypertonic buffer to the surrounding medium or by replacing the surrounding medium by isotonic buffer. The drug to be loaded can be added by either dissolving the drug in isotonic cell suspending buffer inside a dialysis bag at the beginning of the experiment or by adding the drug to a dialysis bag after the stirring is complete . The use of standard hemodialysis equipment for loading a drug in erythrocytes was reported by Roper et al.. In this method, the erythrocyte suspension and the drug to be loaded were placed in the blood compartment and the hypotonic buffer was placed in a receptor compartment.

This led to the concept of “continuous flow dialysis,” which has been used by several other researchers. The loaded cells exhibit the same circulation half life as that of normal cells . Also, this method has high entrapment efficiency on the order of 30–50% , cell recovery of 70–80%, high-loading capacity , and is amenable to automation with control of process variables . The drawbacks include a long processing time and the need for special equipment.
This method has been used for loading enzymes such as β- galactosidase, glucoserebrosidase , asparginase , inositol hexaphosphatase as well as drugs such as gentamicin , adriamycin , pentamidine and furamycin , interlukin-2 ,desferroxamine , and human recombinant erythropoietin. (Gothoskar 2004)

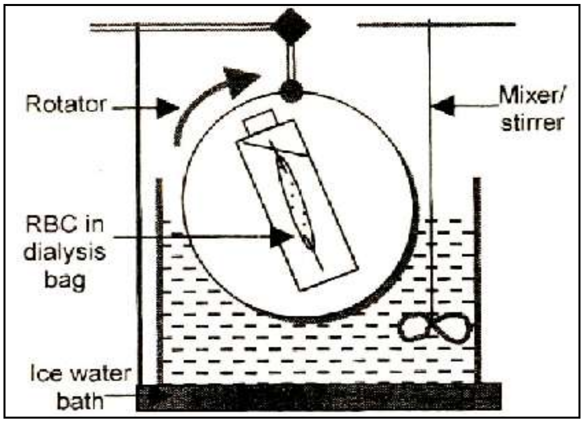

FIG 2: ***HYPOTONIC DIALYSIS*** (rajendra 2011)

7. Isotonic osmotic lysis:

This method, also known as the osmotic pulse method, involves isotonic hemolysis that is achieved by physical or chemical means. The isotonic solutions may or may not be isotonic. If erythrocytes are incubated in solutions of a substance with high membrane permeability, the solute will diffuse into the cells because of the concentration gradient. This process is followed by an influx of water to maintain osmotic equilibrium. Chemicals such as urea solution, polyethylene glycol, and ammonium chloride have been used for isotonic hemolysis. However, this method also is not immune to changes in membrane structure composition. In 1987, Franco et al. developed a method that involved suspending erythrocytes in an isotonic solution of dimethyl sulfoxide (DMSO). The suspension was diluted with an isotonic-buffered drug solution. After the cells were separated, they were resealed at 37°C. (Gothoskar 2004)

8. Chemical perturbation of the membrane:

This method is based on the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemicals. In 1973, Deuticke et al. showed that the permeability of erythrocytes' membrane increases upon exposure to polyene antibiotic such as amphotericin B. In 1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes. Lin et al. used halothane for the same purpose. However, these methods induce irreversible destructive changes in the cell membrane and hence are not very popular. (Gothoskar 2004)

9. Electro-insertion or electro encapsulation :

This method is also known as electroporation, the method consist of creating electrically induced permeability changes at high membrane potential differences. In 1977, Tsong and Kinosita suggested the use of transient electrolysis to generate desirable membrane permeability for drug loading. Electrical breakdown is achieved by membrane polarization for microseconds using varied voltage of 2kv/cm is applied for 20 µsec. The potential difference across the membrane is built up either directly by inter and intracellular electrodes or indirectly by applying internal electric field to the cells. The extent of pore formation depends upon the electric field strength, pulse duration and ionic strength of suspending medium. Once membrane is perforated, regardless of the size of pores, ions rapidly distribute between the extra and intracellular space to attain Donnan equilibrium, however the membrane still remains impermeable to its cytoplasmic macromolecules. The cell membrane eventually lyses because of the colloidal osmotic pressure of its macromolecular contents. In the case of red blood cells, the colloidal osmotic pressure of hemoglobin is about 30 mOsm.

This pressure drives water and ion influx, as a result swelling of the cells occurs. The membrane is ruptured when the cell volume reaches 155% of its original volume. Thus, cell lysis is a secondary effect of electric modification of the membrane. Since the cell lysis is due to colloidal osmotic swelling, the rational to prevent lysis is to balance the colloidal osmotic pressure of cellular macromolecules. This can be affected by addition of large molecules (like tetrasaccharide stachyose or protein such as bovine serum albumin) and ribonucleases. This helps to counteract the colloidal osmotic swelling of electrically perforated erythrocytes. Under this osmotically balanced condition pores stay open at 4°C for few days. If drug molecules are added at this point, they permeate into red blood cells.

A suitable procedure could be subsequently used to reseal these pores. The various candidates entrapped by this method include primaquine and related 8–amino–quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine, and vitamin A. (Gothoskar 2004)

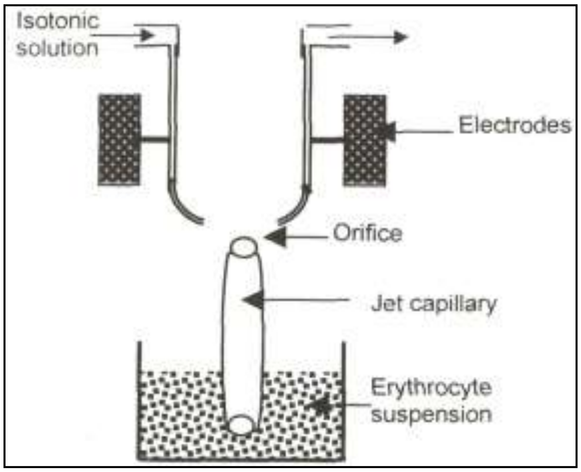

FIG 3: ***Electro-insertion OR***
Electro encapsulation (rajendra 2011)

10.Entrapment by endocytosis :

This method was reported by Schrier et al. in 1975 (Energized Endocytosis in Human Erythrocyte Ghosts 1975)Endocytosis involves the addition of one volume of washed packed erythrocytes to nine volumes of buffer containing 2.5 mM ATP, 2.5 mM MgCl2, and 1mM CaCl2, followed by incubation for 2 min at room temperature. The pores created by this method are resealed by using 154 mM of NaCl and incubation at 37 °C for 2 min. The entrapment of material occurs by endocytosis. The vesicle membrane separates endocytosed material from cytoplasm thus protecting it from the erythrocytes and vice-versa. The various candidates entrapped by this method include primaquine and related 8–amino–quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine, and vitamin A. (Gothoskar 2004)

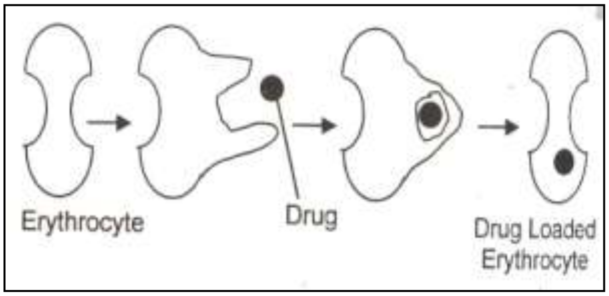

FIG 4: ***Entrapment by endocytosis*** (rajendra 2011)

11. Loading by electric cell fusion :

This method involves the initial loading of drug molecules into erythrocyte ghosts followed by adhesion of these cells to target cells. The fusion is accentuated by the application of an electric pulse, which causes the release of an entrapped molecule. An example of this method is loading a cell-specific monoclonal antibody into an erythrocyte ghost . An antibody against a specific surface protein of target cells can be chemically cross-linked to drug-loaded cells that would direct these cells to desired cells. (Gothoskar 2004)

12. Loading by lipid fusion :

Lipid vesicles containing a drug can be directly fused to human erythrocytes, which lead to an exchange with a lipid-entrapped drug. This technique was used for entrapping inositol monophosphate to improve the oxygen carrying capacity of cells. However, the entrapment efficiency of this method is very low (~ 1%). (Gothoskar 2004)

CHARACTERIZATION OF RESEALED ERYTHROCYTES:

Table I summarizes the various evaluation parameters and the techniques applied for their determination. (Sha 2011)

Drug content quantification:

To determine the drug content, packed loaded cells are deproteinized with acetronitrile after cetifugation at 3000 rpm for a fixed time interval. The clear supernatant liquid is analyzed spectrophotometrically. (Sha 2011)

In-vitro drug release and hemoglobin content study:

In-vitro release of drug(s) and hemoglobin are monitored periodically form drug-loaded cells. The cell suspension(5% hematocrit in PBS) is stored at 4 ^{0}C in amber colored glass containers. Periodically the clear supernatant are withdrawn using a hypodermic syringes equipped with 0.45 m filter, deproteinied using methanol and were estimated for drug content. (Sha 2011)

The supernatant of each sample after centrifugation is collected and assayed, % hemoglobin release may be calculated using the formula. (Li 1996)

$$\%\ \text{hemoglobin release} = \frac{\text{A540 of sample - A540 of background}}{\text{A540 of 100\% hemoglobin}}$$

Or

$$\text{Mean corpuscular hemoglobin \{MCH (pg)\}} = \frac{\text{Hemoglobin (g/100ml) x 10}}{\text{Erythrocyte count (millions/cu mm)}}$$

Where an A540 refers to absorbance at 540nm.

Percent cell recovery and Morphological study:

Percent cell recovery may be determined by counting the no. of intact cells per cubic mm of packed erythrocytes before and after loading the drug. Phase contrast or electron microscope may be used for normal and drug loaded erythrocytes . (Sha 2011)

Osmotic fragility and Osmotic shock study:

When red blood cells are exposed to solution of varying tonicities their shape change due to osmotic imbalance. To study the effect of different tonicities, drug loaded erythrocytes are incubated separately in normal saline solution at 37= ± 2 °C for 10 minutes, followed by centrifugation at 2000 rpm for 10 min. For osmotic shock study, dispersing the resealed erythrocyte suspension in distilled water and centrifuged at 300 rpm for 15 min. The supernatant was estimated for percent hemoglobin release spectrophotometrically. (Sha 2011)

Turbulence shock study:

It is the measure of simulating destruction of loaded cells during injection. Normal and drug loaded cells are passed through a 23 gauge hypodermic needle at a flow rate of 10 ml/min which is comparable to the flow rate of blood. It is followed by collection of an aliquot and centrifugation at 2000 rpm for 10 minutes. The hemoglobin in withdrawn sample is estimated.

Drug loaded erythrocytes appear to be less resistant to turbulence, probably indicating destruction of cells upon shaking . (Sha 2011)

Determination of entrapped magnetite :

Atomic Absorption spectroscopic method is reported for determination of the concentration of a particular metal element in a sample. The HCl is added to a fixed amount of magnetite bearing erythrocytes and content are heated at 60oC for 2hours.then 20%w/v trichloro acetic acid is added and supernatant obtained after centrifugation is used to determine magnetite concentration using atomic absorption spectroscopy. (Shah 2011)

Erythrocyte sedimentation rate(ESR):

It is an estimate of the suspension stability of RBC in plasma and is related to the number and size of the red cells and to relative concentration of plasma protein , especially fibrinogen and α ,β globulins. This test is performed by determining the rate of sedimentation of blood cells in a standard tube. normal blood ESR is 0 to 15 mm/hr. higher rate is indication of active but obscure disease processes. (Shah 2011)

Miscellaneous:

Resealed erythrocyte can also be characterized by cell sizes, mean cell volume ,energy metabolism, lipid composition ,membrane fluidity , rheological properties ,density gradient separation (Shah 2011)

IN VITRO STORAGE:

The success of resealed erythrocytes as a drug delivery system depends to a greater extent on their in vitro storage. Preparing drug-loaded erythrocytes on a large scale and maintaining their survival and drug content can be achieved by using suitable storage methods. However, the lack of reliable and practical storage methods has been a limiting factor for the wide-spread clinical use of the carrier erythrocytes. The most common storage media include Hank's balanced salt solution and acid–citrate–dextrose at 4^0C. Cells remain viable in terms of their physiologic and carrier characteristics for at least 2 weeks at this temperature. The addition of calcium-chelating agents or the purine nucleosides improve circulation survival time of cells upon reinjection. Exposure of resealed erythrocytes to membrane stabilizing agents such as dimethyl sulfoxide, dimethyl,3,3-di-thio-bispropionamide, gluteraldehyde, toluene-2-4-diisocyanate followed by lyophilization or sintered glass filtration has been reported to enhance their stability upon storage . The resultant powder was stable for at least one month without any detectable changes. But the major disadvantage of this method is the presence of appreciable amount of membrane stabilizers in bound form that remarkably reduces circulation survival time. Other reported methods for improving storage stability include encapsulation of a prodrug that undergoes conversion to the parent drug only at body temperature, high glycerol freezing technique , and reversible immobilization in alginate or gelatin gels . (Gothoskar 2004)

IN VIVO LIFE SPAN:

The efficacy of resealed erythrocytes is determined mainly by their survival time in circulation upon reinjection. For the purpose of sustained action, a longer life span is required, although for delivery to target-specific RES organs, rapid phagocytosis and hence a shorter life span is desirable. The life span of resealed erythrocytes depends upon its size, shape, and surface electrical charge as well as the extent of hemoglobin and other cell constituents lost during the loading process. The various methods used to determine in vivo survival time include labeling of cells by 51Cr or fluorescent markers such as fluorescin isothiocyanate or entrapment of 14C sucrose or gentamicin . The circulation survival kinetics of resealed erythrocytes show typical bimodal behavior with a rapid loss of cells during the first 24 h after injection, followed by a slow decline phase with a half life on the order of days or weeks. The early loss accounts for ~15–65% loss of total injected cells . The erythrocytes' carriers constructed of red blood cells of mice, cattle, pigs, dogs, sheep, goats, and monkeys exhibit a comparable circulation profile with that of normal unloaded erythrocytes. On the other hand, resealed erythrocytes prepared from red blood cells of rabbits, chickens, and rats exhibit relatively poor circulation profile. (Gothoskar 2004)

APPLICATIONS OF RESEALED ERYTHROCYTES: (Gothoskar 2004)

Resealed erythrocytes have several possible applications in various fields of human and veterinary medicine. Such cells could be used as circulating carriers to disseminate a drug within a prolonged period of time in circulation or in target-specific organs, including the liver, spleen, and lymph nodes. A majority of the drug delivery studies using drug-loaded erythrocytes are in the preclinical phase. In a few clinical studies, successful results were obtained.

Slow drug release:

Erythrocytes have been used as circulating depots for the sustained delivery of antineoplastics , antiparasitics , veterinary antiamoebics , vitamins , steroids , antibiotics, and cardiovascular drugs .

The various mechanisms proposed for drug release include

- passive diffusion
- specialized membrane associated carrier transport
- phagocytosis of resealed cells by macrophages of RES, subsequent accumulation of drug into the macrophage interior, followed by slow release .
- accumulation of erythrocytes in lymph nodes upon subcutaneous administration followed by hemolysis to release the drug .

Routes of administration include intravenous, which is the most common, followed by subcutaneous, intraperitoneal, intranasal, and oral. Studies regarding the improved efficacy of various drugs given in this form in animal models have been published. Examples include an enhancement in anti-inflammatory effect of corticosteroids in experimentally inflamed rats , increase in half life of isoniazid , levothyroxine , cytosine arabinoside , and interleukin- 2; prolongation of plasma half life of erythropoietin from 30 min to 35 h in mice, and can increase in mean survival time of mice with experimental hepatoma after injecting methotrexate loaded erythrocytes . Thalasemic patients, because of multiple blood transfusions, are prone to hemosydrosis, a disease state associated with an excess storage of iron . This state is treated using SC or IV injections of iron-chelating compound desferrioxamine, which causes severe adverse effects in case of multiple injections. This agent was loaded on to erythrocytes and the performance of these cells upon reinjection was observed and found to be promising. This therapeutic method is approved in the United States as regular management tool of hemosydrosis since 1984.

Drug targeting:

Ideally, drug delivery should be site-specific and target-oriented to exhibit maximal therapeutic index with minimum adverse effects. Resealed erythrocytes can act as drug carriers and targeting tools as well. Surface-modified erythrocytes are used to target organs of mononuclear phagocytic system/ reticuloendothelial system because the changes in the membrane are recognized by macrophages. However, resealed erythrocytes also can be used to target organs other than those of RES.

Targeting RES organ :

Damaged erythrocytes are rapidly cleared from circulation by phagocytic Kupffer cells in liver and spleen. Resealed erythrocytes, by modifying their membranes, can therefore be used to target the liver and spleen. (Franco 1998) The various approaches to modify the surface characteristics of erythrocytes include

- surface modification with antibodies
- surface modification with gluteraldehyde
- surface modification with carbohydrates such as sialic acid
- surface modification with sulphydryl
- surface chemical cross-linking e.g. delivery of 125I-labeled carbonic
anhydrase loaded in erythrocytes cross-linked with *bis*(sulfosuccinimidyl) suberate and 3,3_ dithio (sulfosuccinmidyl propionate) . (Alvarez 1998)

Targeting the liver Enzyme deficiency/replacement therapy:

Many metabolic disorders related to deficient or missing enzymes can be treated by injecting these enzymes. (Jaitely 1996) However, the problems of exogenous enzyme therapy include a shorter circulation half life of enzymes, allergic reactions, and toxic manifestations. These problems can be successfully overcome by administering the enzymes as resealed erythrocytes. The enzymes used include β-glucosidase, β-glucoronidase, β-galactosidase. The disease caused by an accumulation of glucocerebrosides in the liver and spleen can be treated by glucocerebrosidase- loaded erythrocytes.

Treatment of hepatic tumors:

Hepatic tumors are one of the most prevalent types of cancer. Antineoplastic drugs such as methotrexate , bleomycin, asparginase , and adriamycin have been successfully delivered by erythrocytes. Agents such as daunorubicin diffuse rapidly from the cells upon loading and hence pose a problem. This problem can be overcome by covalently linking daunorubicin to the erythrocytes’ membrane using gluteraldehyde or cisaconitic acid as a spacer. The resealed erythrocytes loaded with carboplatin show localization in liver.

Treatment of parasitic diseases:

The ability of resealed erythrocytes to selectively accumulate within RES organs make them useful tool during the delivery of antiparasitic agents. Parasitic diseases that involve harboring parasites in the RES organs can be successfully controlled by this method. Results were favorable in studies involving animal models for erythrocytes loaded with antimalarial, antileishmanial, and antiamoebic drugs.

Removal of RES iron overload:

Desferrioxamine-loaded erythrocytes have been used to treat excess iron accumulated because of multiple transfusions to thalassemic patients. Targeting this drug to the RES is very beneficial because the aged erythrocytes are destroyed in RES organs, which results in an accumulation of iron in these organs.

Removal of toxic agents:

Cannon et al. reported inhibition of cyanide intoxication with murine carrier erythrocytes containing bovine rhodanase and sodium thiosulfate . Antagonization of organophosphorus intoxication by resealed erythrocytes containing a recombinant phosphodiestrase also has been reported .

Targeting organs other than those of RES:

Recently, resealed erythrocytes have been used to target organs outside the RES. The various approaches include

- entrapment of paramagnetic particles along with the drug
- entrapment of photosensitive material
- the use of ultrasound waves
- antibody attachment to erythrocyte membrane to get specificity of action

Zimmermann (U 1975) proposed that the entrapment of small paramagnetic particles into erythrocytes might allow their localization to a particular location under the influence of an external magnetic field. The loading of ferrofluids (colloidal suspension of magnetite) has been reported by Sprandel et al. Jain and Vyas (Jain and Vyas 1994) reported entrapment of the anti-inflammatory drugs diclofenac sodium and ibuprofen in magnetoresponsive erythrocytes. Photosensitized erythrocytes have been studied as a photo triggered carrier and delivery system for methotrexate in cancer treatment . Chiarantini et al. have reported in vitro targeting of erythrocytes to cytotoxic T-cells by coupling of Thy-1.2 monoclonal antibody . Price et al. reported delivery of colloidal particles and erythrocytes to tissue through micro vessel ruptures created by targeted micro bubble destruction with ultrasound. IV fluorescent erythrocytes were delivered to the interstitium of rat skeletal muscle through micro vessel ruptures by insonifying micro bubbles in vivo. This technique provides a noninvasive means for delivering resealed erythrocytes across the endothelial carrier to the target tissue. Other approaches for targeting organs outside the RES include the preparation of carrier erythrocytes fused to thermo responsive liposomes and their localization using an external thermal source, intraperitoneal injection of resealed erythrocytes for drug targeting to peritoneal macrophages, and lectin pretreatment of resealed cells loaded with antineoplastic drugs to improve targeting tumor cells .

Delivery of antiviral agents:

Several reports have been cited in the literature about antiviral agents entrapped in resealed erythrocytes for effective delivery and targeting . Because most antiviral drugs are either nucleotides or nucleoside analogs, their entrapment and exit through the membrane need careful consideration. Nucleosides are rapidly transported across the membrane whereas nucleotides are not, and thus exhibiting prolonged release profiles. The release of nucleotides requires conversion of these moieties to purine or pyrimidine bases. Resealed erythrocytes have been used to deliver deoxycytidine derivatives, recombinant herpes simplex virus type 1 (HSV-1) glycoprotein B , azidothymidine derivatives , azathioprene, acyclovir , and fludarabine phosphate .

Enzyme therapy:

Enzymes are widely used in clinical practice as replacement therapies to treat diseases associated with their deficiency (e.g., Gaucher's disease, galactosuria), degradation of toxic compounds secondary to some kind of poisoning (cyanide, organophosphorus), and as drugs . The problems involved in the direct injection of enzymes into the body have been cited. One method to overcome these problems is the use of enzyme-loaded erythrocytes. These cells then release enzymes into circulation upon hemolysis ; act as a "circulating bioreactors" in which substrates enter into the cell, interact with enzymes, and generate products; or accumulate enzymes in RES upon hemolysis for future catalysis. The first report of successful clinical trials of the resealed erythrocytes loaded with enzymes for replacement therapy is that of β-glucoserebrosidase for the treatment of Gaucher's disease . The disease is characterized by inborn deficiency of lysosomal β-glucoserebrosidase in cells of RES thereby leading to accumulation of β-glucoserebrosides in macrophages of the RES.

The most important application of resealed erythrocytes in enzyme therapy is that of asparginase loading for the treatment of pediatric neoplasms. This enzyme degrades aspargine, an amino acid vital for cells. This treatment prevents remission of pediatric acute lymphocytic leukemia. There are reports of improved intensity and duration of action in animal models as well as humans.

To treat lead poisoning, the concentration of β-aminolevulinate dehydrogenase (ALA-D) in erythrocytes decreases. This leads to an accumulation of β-aminolevulinic acid in tissues, blood, and urine. This state leads to acute porphyria and CNS related problems . An injection of resealed erythrocytes loaded with ALA–D to lead intoxicated animal significantly reduces toxic manifestations. Other enzymes used for loading resealed erythrocytes include urease, galactose-1-phosphate uridyl transferase, uricase, and acetaldehyde dehydrogenase.

Improvement in oxygen delivery to tissues:

Hemoglobin is the protein responsible for the oxygen-carrying capacity of erythrocytes. Under normal conditions, 95% of hemoglobin is saturated with oxygen in the lungs, whereas under physiologic conditions in peripheral blood stream only~25% of oxygenated hemoglobin becomes deoxygenated. Thus, the major fraction of oxygen bound to hemoglobin is recirculated with venous blood to the lungs. The use of this bound fraction has been suggested for the treatment of oxygen deficiency. 2,3-Diphosphoglycerate (2,3-DPG) is a natural effector of hemoglobin. The binding affinity of hemoglobin for oxygen changes reversibly with changes in intracellular concentration of 2,3-DPG. This compensates for changes in the oxygen pressure outside of the body, as the affinity of 2,3-DPG to oxygen is much higher than that of hemoglobin .

Other organic polyphosphates can serve as allosteric effectors of hemoglobin with binding affinities higher than those of 2,3-DPG and can compete with 2,3-DPG for binding to hemoglobin. Inositol hexophosphate (IHP) is one of the strongest effectors of this type. However, because of its ionization at physiologic pH, it cannot enter erythrocytes. Hence, it is entrapped by the electroporation process. Upon encapsulation, IHP irreversibly binds to hemoglobin, thereby decreasing the oxygen affinity to hemoglobin and subsequent shift of oxygen binding isotherm to the right. As a result, the oxygen pressure corresponding to 50% of the total binding capacity of hemoglobin to oxygen (P50 value) increases from 26–27 mm Hg to >50 mm Hg .

In the presence of IHP encapsulated in erythrocytes, the difference between the oxygen bound fraction of hemoglobin in lungs and tissues increases, thereby increasing the oxygen concentration in tissues. Also, the extent of carbamate formed in the N-terminal amine group of βchain of hemoglobin decreases, which is compensated by an uptake of H^+ and CO2 that leads to increased formation of bicarbonate ion.

IV injection of IHP-loaded erythrocytes to piglets led to a decrease in cardiac output with a constant oxygen consumption by animals. This indicates that because of an increased extraction ratio of oxygen by tissues, a given amount of oxygen can be delivered in lower blood flow. In addition, these erythrocytes reduce ejection fraction, left ventricular diastolic volume, and heart rate .An isolated perfused-heart model showed reduction in coronary blood flow with increased oxygen consumption by myocardium upon administration of IHP-loaded erythrocytes . The same results are reported when intact animal models were used. Application of IHP-loaded erythrocytes for improved oxygen supply is beneficial under the following conditions:

- high altitude conditions where the partial pressure of oxygen is low
- reduction in the number of alveoli, where exchange surface of the lungs is decreased
- increased resistance to oxygen diffusion in the lungs
- reduction in oxygen transport capacity
- mutation or chemical modification, which involves a decrease in oxygen affinity for hemoglobin
- increased radio sensitivity of radiation-sensitive tumors
- restoration of oxygen-delivery capacity of stored blood
- ischemia of myocardium, brain, or other tissues .

MICROINJECTION OF MACROMOLECULES:

Biological functions of macromolecules such as DNA, RNA, and proteins are exploited for various cell biological applications. Hence, various methods are used to entrap these macromolecules into cultured cells (e.g., microinjection). A relatively simple structure and a lack of complex cellular components (e.g., nucleus) in erythrocytes make them good candidates for the entrapment of macromolecules. In microinjection, erythrocytes are used as micro syringes for injection to the host cells.

The microinjection process involves culturing host eukaryotic cells in vitro. The cells are coated with fusogenic agent and then suspended with erythrocytes loaded with the compound of interest in an isotonic medium. Sendai virus (hemagglutinating virus of Japan, HVJ) or its glycoprotein's or polyethylene glycol have been used as fusogenic agents. The fusogen causes fusion of cosuspended erythrocytes and eukaryotic cells. Thus, the contents of resealed erythrocytes and the compound of interest are transferred to host cell. This procedure has been used to microinject DNA fragments , arginase , proteins, nucleic acids, ferritin, latex particles, bovine and human serum albumin, and enzyme thymidine kinase to various eukaryotic cells.

Advantages of this method include quantitative injection of materials into cells, simultaneous introduction of several materials into a large number of cells, minimal damage to the cell, avoidance of degradation effects of lysosomal enzymes, and simplicity of the technique. Disadvantages include a need for a larger size of fused cells, thus making them amenable to RES clearance, adverse effects of fusogens, unpredictable effects on cell resulting from the cointroduction of various components. Hence, this method is limited to mainly cell biological applications rather than drug delivery. (Gothoskar 2004)

NOVEL APPROACHES:

Erythrosomes:

These are specially engineered vesicular systems that are chemically cross-linked to human erythrocytes support upon which a lipid bilayer is coated. (Legha 1981) This process is achieved by modifying a reverse-phase evaporation technique. These vesicles have been proposed as useful encapsulation systems for macromolecular drugs. (rajendra 2011)

Nanoerythrosomes:-

These are prepared by extrusion of erythrocyte ghosts to produce small vesicles with an average diameter of 100 nm. Daunorubicin was covalently conjugated to nanoerythrosomes using gluteraldehyde spacer. (Moorjani 1996) This complex was more active than free daunorubicin alone, both in vitro and in vivo. (rajendra 2011)

FUTURE PERSPECTIVES:

The concept of employing erythrocytes as drug or bio active carrier still needs further optimization a large amount of valuable work is needed so us to utilize the potential of erythrocyte in passive and as well as active targeting of drugs. Disease like cancer would surely find it cure. Genetic engineering aspects can be coupled to give a newer dimension to the existing cellular drug concept. (Gothoskar 2004)

TABLE 1: VARIOUS CHARACTERIZATION PARAMETERS AND METHOD EMPLOYED FOR RESEALED ERYTHROCYTES (Sha 2011)

PARAMETER		**INSTRUMENT/METHOD USED**
I.	**Physical parameter**	
a)	Shape, size, surface morphology	Transmission electron microscopy, scanning electron microscopy, optical microscopy, phase contrast microscopy.
b)	Vesicle size and size distribution	Transmission electron microscopy, optical microscopy
c)	Drug release	Diffusion cell, dialysis
d)	Drug content	Deproteinization of cell membrane followed by assay of drug, radio labelling
e)	Surface electrical potential spectroscopy	Zeta potential determination by photon correlation(PCS)
f)	Surface pH	Ph sensitive probes
g)	deformity	Capillary method
II.	**Cellular parameter**	
a)	% Hb content	Deproteinization of cell membrane followed by haemoglobin assay of drug
b)	Cell volume	Laser light scattering
c)	% cell recovery	Neubaur's chamber, haematological analyser
d)	Osmotic fragility	Stepwise incubation with isotonic to hypotonic saline solutions and determination of drug and haemoglobin assay
e)	Osmotic shock	Dilution with distilled water and estimation of drug and haemoglobin
f)	Turbulent shock	Passage of cell suspension to 30-gauge hypodermic needle at the rate of 10ml/min flow and estimation of residual drug and haemoglobin, vigorous shaking
g)	Erythrocyte sedimentation rate	Determine by ESR technique
III.	**Biological parameter**	
a)	Pyrogenicity	LAL test, rabbit method
b)	Sterility	Sterility testing method
c)	toxicity	Toxicity test method

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PARTICULATE DRUG DELIVERY SYSTEMS - MONOCLONAL ANTIBODIES

Suryavanshi Kiran, Mogal Rajendra

INTRODUCTION:

Monoclonal antibody therapy is the use of monoclonal antibodies (or mAb) to specifically bind to target cells or proteins. This may then stimulate the patient's immune system to attack those cells. It is possible to create a mAb specific to almost any extracellular/ cell surface target. In essence, they can be deployed to find a single targeted substance, such as an antigen found only on a cancer cell, and make it possible to pinpoint the cell and destroy it. In addition to cancer therapies, MAbs are also used in diagnostic tests for everything from pregnancy, to AIDS, to drug screening. Further, the antibodies can be used to lessen the problem of organ rejection in transplant patients and to treat viral diseases that are traditionally considered "untreatable."

Monoclonal antibody technology allow us to produce large amounts of pure antibodies obtaining cells that produce antibodies naturally, in effect having a factory to produce antibodies that worked around the clock. Two biotechnology techniques have been important in creating and producing MAbs: cell fusion and cell culture. Early therapeutic MAbs were made from mice, but human bodies rejected them. Modern genetic engineering allows developers to "humanize" the antibodies. Often they are called "chimeric" - chimeras were mythological creatures that mixed parts of two or more real animals together.

ORIGIN OF MONOCLONAL ANTIBODIES:

Immunotherapy developed as a technique with the discovery of the structure of antibodies and the development of hybridoma technology, which provided the first reliable source of antibodies. These advances allowed for the specific targeting of tumors both in vitro and in vivo. Initial research on malignant neoplasms found MAb therapy of Limited and generally short-lived success with malignancies of the blood. Furthermore treatment had to be specifically tailored to each individual patient, thus proving to be impracticable for the routine clinical setting.

Throughout the progression of monoclonal drug development there have been four major antibody types developed: murine, chimeric, humanized and human. Initial therapeutic antibodies were simple murine analogues, which contributed to the early lack of success. It has since been shown that these antibodies have: a short half-life in vivo (due to complex formation), limited penetration into tumor sites, and those they inadequately recruit host effector functions. To overcome these difficulties the technical issues initially experienced had to be surpassed. Chimeric and humanized antibodies have generally replaced murine antibodies in modern therapeutic antibody applications. Hybridoma technology has been replaced by recombinant DNA technology, transgenic mice and phage display. Understanding of proteomics has proven essential in identifying novel tumor targets. (wikipwdia.com n.d.)

Structure of Monoclonal antibodies:

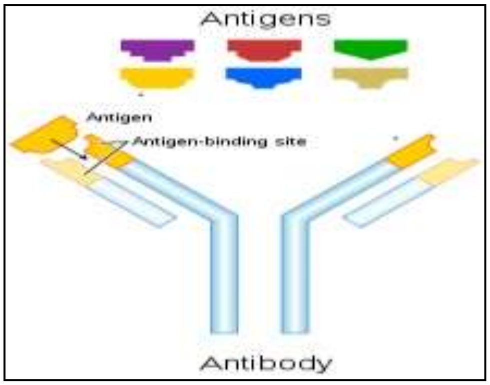

Figure no. 1: Structure of Monoclonal antibody (wikipedia.com n.d.)

Antibodies are large heterodimeric molecules, approximately 150 kDa and are composed of two different kinds of polypeptide chain, called the heavy (~50kDa) and the light chain (~25kDa). There are two types of light chains, kappa (κ) and lambda (λ). By cleavage with enzyme papain, the Fab (*fragment-antigen binding*) part can be separated from the Fc (*fragment constant*) part of the molecule (see image). The Fab fragments contain the variable domains, which consist of three hypervariable amino acid domains responsible for the antibody specificity embedded into constant regions.

The immune system responds to the environmental factors it encounters on the basis of discrimination between self and non-self. Tumor cells are not specifically targeted by one's immune system since tumor cells are the patient's own cells. Tumor cells, however are highly abnormal, and many display unusual antigens that are either inappropriate for the cell type, its environment, or are only normally present during the organisms' development (e.g. fetal antigens). Other tumor cells display cell surface receptors that are rare or absent on the surfaces of healthy cells, and which are responsible for activating cellular signal transduction pathways that cause the unregulated growth and division of the tumor cell. Examples include ErbB2, a constitutively active cell surface receptor that is produced at abnormally high levels on the surface of approximately 30% of breast tumor cells. Such breast cancer is known a HER2 positive breast cancer.

Antibodies are a key component of the adaptive immune response, playing a central role in both in the recognition of foreign antigens and the stimulation of an immune response to them. The advent of monoclonal technology has made it possible to raise antibodies against specific antigens presented on the surfaces of tumors. (wikipwdia.com n.d.)

MECHANISM OF ACTION:

Monoclonal antibodies achieve their therapeutic effect through various mechanisms. They can have direct effects in producing apoptosis or programmed cell death. They can block growth factor receptors, effectively arresting proliferation of tumor cells. In cells that express monoclonal antibodies, they can bring about anti-idiotype antibody formation.

Indirect effects include recruiting cells that have cytotoxicity, such as monocytes and macrophages. This type of antibody-mediated cell kill is called antibody-dependent cell mediated cytotoxicity (ADCC). Monoclonal antibodies also bind complement, leading to direct cell toxicity, known as complement dependent cytotoxicity (CDC).

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)] in this situation, a chimeric antibody, targets the CD20 antigen. This antigen is expressed on a significant number of B cell malignancies. The Fc fragment of the monoclonal antibody binds the Fc receptors found on monocytes, macrophages, and natural killer cells. These cells in turn engulf the bound tumor cell and destroy it. Natural killer cells secrete cytokines that lead to cell death, and they also recruit B cells

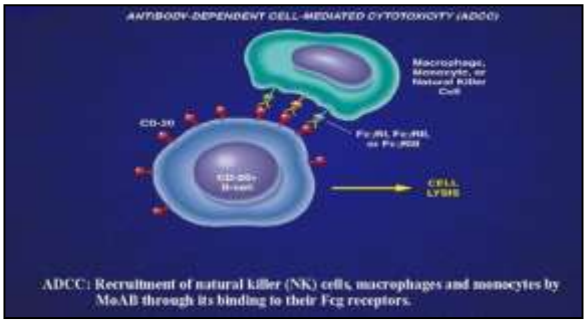
ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY (ADCC)
Macrophage, Monocyte, or Natural Killer Cell
CD-20
FcγRI, FcγRII, or FcγRIII
CD-20+ B-cell
CELL LYSIS
ADCC: Recruitment of natural killer (NK) cells, macrophages and monocytes by MoAB through its binding to their Fcg receptors.

Figure no.2 Antibody Dependent Cell Mediated Cytotoxicity (Anderson DR et al n.d.)

An example of complement dependent cytotoxicity, again using B cell lymphoma, is shown in Figure 2. [Figure 2] Complement-Dependent Cytotoxicity (CDC).Here we see the monoclonal antibody binding to the receptor and initiating the complement system, also known as the 'complement cascade.' The end result is a membrane attack complex that literally makes a hole within the cell membrane, causing cell lysis and death.

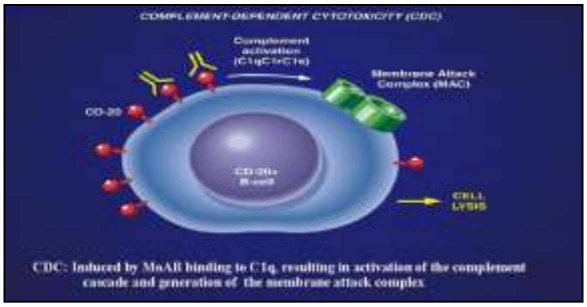
COMPLEMENT-DEPENDENT CYTOTOXICITY (CDC)
Complement activation (C1qC1rC1s)
Membrane Attack Complex (MAC)
CD-20
CD-20+ B-cell
CELL LYSIS
CDC: Induced by MoAB binding to C1q, resulting in activation of the complement cascade and generation of the membrane attack complex

Figure no.3 Cell Dependent Cytotoxicity (Anderson DR et al n.d.)

PRODUCTION OF MONOCLONAL ANTIBODIES:

Monoclonal antibodies are antibodies which have a single, selected specificity and which are continuously secreted by immortalized hybridoma cells. A hybridoma is a biologically constructed hybrid of a mortal, antibody-producing, lymphoid cell, and a malignant, or immortality, myeloma cell. Following the discovery of hybridoma technology in 1975, developments in mAb production and in their application have had profound implications not only on medical research, diagnosis and therapy, but also on biology in general. Hybridoma technology represents a significant advance because, in principle, it provides a means for obtaining unlimited supplies of highly specific antibodies.

In the production of mAbs, animals (generally rats or mice) first have to be immunized with the target antigen to obtain mortal antibody-producing cells. The biological construction of hybrids, and the selection of hybridomas which produce antibodies with the desired specificities, are carried out in vitro. In the early days of hybridoma technology (the late 1970s), the hybridomas developed in vitro were injected into the peritoneal cavity of an animal so that useful amounts of the desired mAb could be harvested from the ascitic fluid. This procedure was considered necessary at the time, since no efficient large-scale in vitro methods were available. By the mid-1980s, there were already serious doubts regarding the necessity of such a painful animal procedure. Nevertheless, as a result of its early introduction as part of the hybridoma technology, ascites production of mAbs is now employed worldwide, in spite of the ongoing development of in vitro technologies and the growing public pressure to replace or reduce animal experiments. There are essentially two stages in the production of mAbs:

a) the induction of antibody producing lymphoid cells in vivo and the selection of antibody-producing hybridoma cells in vitro; and

b) The in vitro/in vivo propagation of selected hybridoma clones.

The first stage, the formation and selection of the hybridoma clone, involves the use of one or more animals (except in rare cases when a human mAb is being developed), and is carried out in the following way:

1. The antigen is injected into mice (or rats). The antigen is often injected in combination with an adjuvant, to enhance the immune response, even though the use of adjuvant generally leads to severe side-effects.
2. After an appropriate interval (5 to 21 days), the immunized animals are killed and lymphoid cells (including progenitor antibody-producing cells) are isolated from the spleen.
3. The lymphoid cells are fused with myeloma cells which have been grown in vitro.
4. The two original cell types and the newly formed hybrids are cultured in a selective medium, such as HAT (hypoxanthine/ aminopterin/thymine) medium, which only allows the hybridoma cells to grow.
5. The supernatant media from the numerous in vitro microcultures exhibiting a recognizable growth of hybridomas are screened for secretion of the desired antibody, by using various immunoassay procedures.
6. The selected cells are sub cultured in vitro, using special cloning procedures to ensure that each in vitro culture consists of hybridomas with single antibody specificity only.
7. Hybridoma cells can be cryopreserved at this stage.

(Uwe Marx 1997)

MONOCLONAL ANTIBODY PRODUCTION IN VIVO:

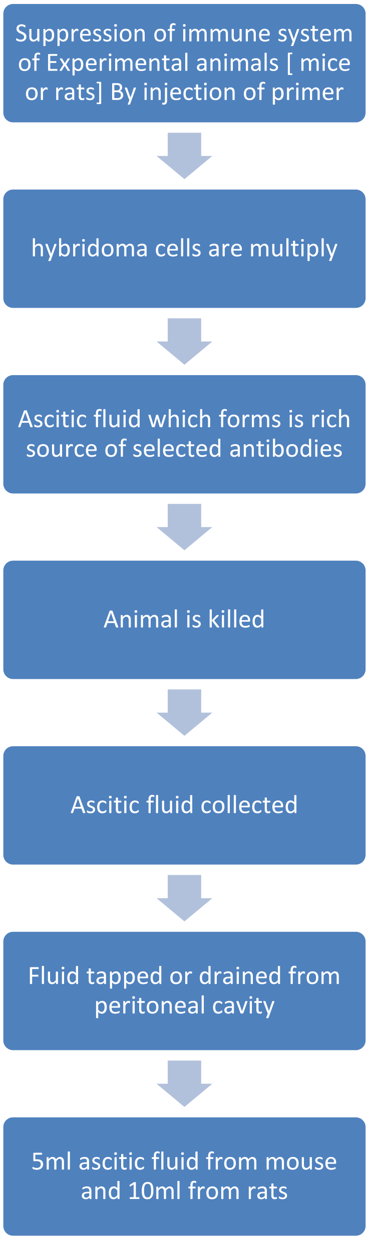
Suppression of immune system of Experimental animals [mice or rats] By injection of primer
hybridoma cells are multiply
Ascitic fluid which forms is rich source of selected antibodies
Animal is killed
Ascitic fluid collected
Fluid tapped or drained from peritoneal cavity
5ml ascitic fluid from mouse and 10ml from rats

Figure no.4: Flow chart of in vivo production of antibodies (Uwe Marx 1997)

ADVANTAGE:

- The main advantage of the ascites method is the extremely high yield of antibody, which generally lies in the range 1-20mg/ml. In addition, the method is not excessively labor-intensive.

DISADVANTAGES

- Extremely painful for the animals used
- Injection of primer;
- The resulting peritonitis caused by the primer
- Abdominal tension; and
- The invasive tumors which result.
- Also a potential risk of product contamination by viruses which are pathogenic to humans.
- the individual batches of harvested ascitic mAb are of variable quality, and they are contaminated with bioreactive cytokines (Uwe Marx 1997)

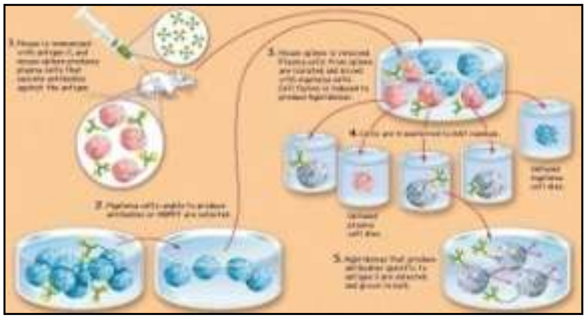

Figure No.5 In Vivo Production of Monoclonal antibodies (Hsu 2011)

In Vitro Production Procedures

In vitro production systems during the last 20 years, a wide range of in vitro production systems have been developed for different purposes. While most of them are useful for the in vitro production of mAbs, they differ in terms of: a) the ease with which they are handled; b) the antibody yield per culture or bioreactor run; and c) the maximum antibody titre achievable. The antibodies produced generally express an immunoreactivity of 90-95%, irrespective of the system used.

Three categories of in vitro production system can be identified according to the principle underlying the culture system:

a) Static and agitated suspension cultures;

b) membrane-based and matrix-based culture systems; and

c) High cell density bioreactors.

Some of these systems have been reviewed recently.

Static and agitated suspension cultures Systems: in this category, which include the widely used T-flasks, roller cultures and spinner cultures, allow the growth of a maximum of two litres of supernatant per culture unit, and a maximum antibody yield of 100-200mg. They are easy to handle in cell culture laboratories, enable various hybridoma cell lines to be propagated simultaneously, and are useful for most of the users in group A.

Investment costs are low because disposable plastic ware is readily available, particularly when using T-flasks. The use of serum-free media, or low-cost additives permitting a reduction in the serum concentration, can greatly reduce costs, while efficiently supporting hybridoma growth 9-13). For example, two serum-free media use a combination of transferring and insulin (9, 10), whereas two low-serum media use a combination of 1% fetal calf serum (FCS) and 0.1% PrimatoneÆ, a peptic digest of animal tissues. This supports hybridoma growth in all culture methods tested at least as efficiently as 5% FCS, at approximately 25% of the cost (M.J. Embleton, personal observation). For the production of mAbs in amounts greater than 100mg, conventional stirred tank bioreactors of different sizes are available. These bioreactors need to be used by specially trained staff and are relevant for user groups B, C and D. The concentration of hybridoma cells in suspension cultures hardly ever exceeds 5 × 106 cells/ml and, in general, the maximum antibody concentration achievable is below 100µg/ml. As a result of the low antibody concentration, the supernatant usually has to be concentrated by ultra filtration if any further purification steps are to be carried out. Feeding of cultures may be carried out periodically if required but, in practice, antibody concentration is increased by 2 to 4 times if the cultures are allowed to grow to exhaustion over 2 to 3 weeks without feeding times, if the cultures are allowed to grow to exhaustion over 2 to 3 weeks without feeding. (Uwe Marx 1997)

Membrane-based and matrix-based culture systems:

This category includes membrane-based and matrix-based static cultures as well as suspension bioreactors. These systems are suitable for user groups A, B and C, which require up to 10g of mAb. In membrane-based systems, the cells are cultured in compartments separated from the nutrient supply by perfusion membranes; special gassing membranes enhance the oxygen transfer into these systems. They produce yields of up to 100mg per culture (user group A), and generate intermediate antibody concentrations of up to 500µg/ml. In addition, they are easy to handle and enable various different cultures to be run simultaneously in routinely equipped cell culture laboratories.

In matrix-based systems, such as fluidized bed or ceramic bioreactors, the immobilization of cells on matrices enables them to be perfused actively and continuously with fresh medium. Irrespective of the size and running time of the bioreactors, 0.1 to 10g of mAbs (user groups B and C) can be produced, corresponding to a maximum concentration of 500µg/ml. In most cases, the supernatant produced has to be concentrated by precipitation or ultra filtration before special purification procedures can be carried out. Special training is required for the proper handling of these systems. (Uwe Marx 1997)

High cell density bioreactors:

This category includes all culture systems which are capable of generating cell densities greater than 108 cells/ml and which, in certain cases, can maintain viable tissue-like cultures. The bioreactors meet the needs of user groups B and C, as they are capable of generating 0.1.10g mAb. The corresponding concentrations lie in the range 0.5.5mg/ml, due to the high cell densities in these systems.

They can be run in conventional cell culture laboratories and models are available for the simultaneous propagation of different cell lines. The product can be used directly or purified without prior concentration. Training is recommended for these systems and is usually provided by the manufacturer. In the most common system within this category, the hollow fibre bioreactor, the culture medium is passed through bundles of hollow fibres, enabling the cell growth compartment to be perfused continuously and effectively. Due to the high antibody concentration, the maximum amount of 500mg of antibody needed by user group B can be produced in a bulk of only 500ml of supernatant, which is easy to handle and process in a conventional cell culture laboratory. Even for user group C, which requires up to 10g of mAb, the total product can be produced in only 10 liters.

The different categories of culture system are listed according to their usefulness to the different user groups in Table I. Instead of the maximum achievable mAb concentration, the concentration which is normally achievable is given. The types of systems recommended for the different user groups, on the basis of their ease of handling, production costs, and advantages with respect to antibody purification. (Uwe Marx 1997)

TYPES OF MONOCLONAL ANTIBODIES:

Murine monoclonal antibodies (suffix *-omab*):

Initially, murine antibodies were obtained by hybridoma technology, for which Kohler and Milstein received a Nobel Prize. However the dissimilarity between murine and human immune systems led to the clinical failure of these antibodies, except in some specific circumstances. Major problems associated with murine antibodies included reduced stimulation of cytotoxicity and the formation complexes after repeated administration, which resulted in mild allergic reactions and sometimes anaphylactic shock.

Chimeric and humanized monoclonal antibodies (suffixes *-ximab*, *zumab* respectively):

To reduce murine antibody immunogenicity, murine molecules were engineered to remove immunogenic content and to increase their immunologic efficiency. This was initially achieved by the production of chimeric and humanized antibodies. Chimeric antibodies are composed of murine variable regions fused onto human constant regions. Human gene sequences, taken from the kappa light chain and the IgG1 heavy chain, results in antibodies that are approximately 65% human. This reduces immunogenicity, and thus increases serum half-life.

Humanized antibodies are produced by grafting murine hypervariable amino acid domains into human antibodies. This results in a molecule of approximately 95% human origin. However it has been shown in several studies that humanised antibodies bind antigen much more weakly than the parent murine monoclonal antibody, with reported decreases in affinity of up to several hundredfold. Increases in antibody-antigen binding strength have been achieved by introducing mutations into the complementarily determining regions (CDR), using techniques such as chain-shuffling, randomization of complementarity determining regions and generation of antibody libraries with mutations within the variable regions by error-prone PCR, E. coli mutator strains, and site-specific mutagenesis.

Human monoclonal antibodies (suffix *-umab*):

Human monoclonal antibodies are produced using transgenic mice or phage display libraries. Human monoclonal antibodies are produced by transferring human immunoglobulin genes into the murine genome, after which the transgenic mouse is vaccinated against the desired antigen, leading to the production of monoclonal antibodies, allowing the transformation of murine antibodies in vitro into fully human antibodies.

The heavy and light chains of human IgG proteins are expressed in structural polymorphic (allotypic) forms. Human IgG allotype has been considered as one of the many factors that can contribute to immunogenecity.
(wikipwdia.com n.d.)

PHARMACOKINETIC PARAMETERS:

Some kinetic studies suffer from a lack of information regarding the assay, the sampling process and the calculation of the parameters. Furthermore, the data are often incomplete and not clearly presented. Concentrations of monoclonal antibodies in biological fluids are generally determined by enzyme-linked immunosorbent assay (ELISA). Radioimmunoassay (RIA) and flow cytometry have also been used. Concerning antibodies conjugated to radionuclides,

pharmacokinetic characteristics are derived from the blood or organ radioactivity content. The sampling duration or the number of time-points often appears insufficient to correctly estimate the elimination parameters, given their potential long half-life (3 weeks, by analogy to endogenous IgG). Hence, some data must be interpreted with caution. In general, monoclonal antibodies exhibit a low volume of distribution (3-5 l), approaching the serum/plasma volume, a low systemic clearance (around 0.5- 4 ml/min) and a terminal half-life varying between 2 days and 28 days. The half-life of murine antibodies is generally shorter than that of antibodies containing a human Fc domain (*i.e.* chimeric, humanised). This could be due to a lesser affinity of the murine Fc domain for the human FcRn. Indeed, preclinical data have indicated that increasing the affinity of the antibody for FcRn could lead to a longer half-life.
(DOMINIQUE LEVÊQUE n.d.)

APPLICATIONS OR USES OF MONOCLONAL ANTIBODIES:

Uses of antibodies. Antibodies have a wide variety of uses.

1. They can be used to immuno-localize a particular antigen in a tissue (immunohistochemistry*). Tissue can be fixed and incubated with the antibodies of interest. These antibodies can then be localized using a 'secondary' antibody coupled to a gold particle or another enzyme that gives a chemical reaction like horse radish peroxidase or beta galactosidase. A secondary antibody is frequently made by generating an immune response to the Fc region of the primary antibody in another species. Thus, if the primary antibody is a mouse IgG, then the secondary could be a rabbit anti-mouse antibody which has been linked to beta galactosidase. Alternatively the antibody can be purified and then conjugated to another molecule to produce a fluorescent antibody.
2. Antibodies can be used to quantitate the presence of an antigen using either a radioimmunoassay* (RIA) or an enzyme-linked immunoabsorbent assay (ELISA)*. There are many variants of these approaches, but those are based on a similar idea. For example, if an antigen can be bound to a solid support or surface, it can be detected by reacting it with a specific antibody and the antibody can be quantitated by reacting it with either a secondary antibody or by incorporating a label directly into the primary antibody. Alternatively, an antibody can be bound to a solid surface and the antigen added. A second antibody that recognizes a distinct epitope on the antigen can then be added and detected. This is frequently called a 'sandwich assay' and can frequently be used to avoid problems of high background or non-specific reactions. It was only an assay of this type, for example that was sensitive and reproducible enough to measure low concentrations of nerve growth factor present in the body.
3. Antibodies can have effects in vivo. These effects can be used in an experimental sense to determine the importance of a particular molecule in vivo. For example, antibodies to nerve growth factor demonstrated that nerve growth factors had to be present for the sympathetic and sensory nervous system to develop. Antibodies to particular hormones or cell-surface receptors can also have a therapeutic use. For example, antibodies to EGF receptor have effects on the development of some tumor types.
4. Since antibodies have a high affinity for a particular epitope, they can be used as affinity reagents in protein purification.
5. Because particular antibodies bind to proteins with high affinity they can also be used as classic criteria for the importance of a particular enzyme or other macromolecule in a particular reaction. If an antibody can interfere with a reaction in a solution that suggests that it may be binding specifically to a protein involved in that reaction. Of course, it must be remembered that some antibodies may be directed against a site on a protein that is not important for enzymatic activity and so may not have the classic "blocking" activity.
6. Antibodies can also be used in conjunction with gel-shift experiments to identify specific DNA-binding proteins.
7. It is also possible to use an antibody to detect a protein after fractionation by SDS-PAGE. This approach, which is called 'western blotting' because it is analogous with northern blotting and Southern blotting, involves transferred a fractionation protein to a nitrocellulose sheet. These proteins can then be exposed to a particular antibody and the antibody

can recognize the protein bound to the blot. This allows the identification of a particular protein..

8. The combination of SDS gels and immunoprecipitation can also be extremely effective. If a particular protein can be immunoprecipitated in a solution, both supernatant and precipitated fractions can be separated on an SDS gel and studied.

- Sometimes an antibody directed against one protein will also precipitate a second protein that has a functional interaction with the first. The second protein, as well as the first, can then be seen by staining the gel or by autoradiography. This is frequently the first indication that a protein functions as part of a complex and it can also be used to demonstrate a physical interaction of two proteins that are hypothesized to interact on the basis of other evidence (e.g., a two hybrid screen* or a suppressor mutation).
- This approach can be combined with western style analysis in several extremely effective ways. An immunoprecipitated protein can be subsequently studied by western analysis* using a different antibody that binds to the protein. The most useful of these antibodies are those that are directed against particular structural determinants that may be present in a protein. Thus, an antibody directed against a region of the protein that undergoes proteolytic processing can be useful to follow proteolytic processing. More commonly, an antibody or a mixture of antibodies that recognize phophorylated peptides (e.g., anti PY (phosphorylated tyrosine) can be used to follow the extent of phosphorylation of a protein (using western analysis) after it is precipitated--or vise-versa. Glycosylation reactions can be followed by antibodies directed against a carbohydrate epitope (or by lectins, which are proteins that recognize carbohydrates).
- Likewise, some antibodies can be made that specifically recognize a phosphorylated epitope, that is they will recognize a tyrosine or a serine after phosphorylation, but not in the absence of phosphate. This is a powerful approach to determine the phosphorylation state of a particular protein. For example, the phosphorylation of CREB (the cAMP response element binding protein) can be followed by an antibody that specifically recognizes an epitope in a way that is dependent on the phosphorylation of serine 133. Thus, the combination of immunoprecipitation and western analysis is an extremely important technique in the study of signal transduction and protein processing.
- Finally, it should be remembered that blotting of a protein gel can be used with reagents other than antibodies. Indeed, some of the first uses of this type of approach were the use of lectins, multivalent carbohydrate-binding proteins, to identify proteins that had been glycosylated on SDS gels. Likewise, some DNA-binding proteins will recognize oligonucleotides after fractionation on SDS gels. This technique is frequently called South-western analysis because of its relationship between both Southern and western analysis.

9. Antibodies, including antisera, can be used to screen expression libraries to isolate candidate genes that express a particular epitope.

10. If a fluorescent antibody is bound to a cells surface it can be used as a marker to quantitate the fraction of cells expressing that marker using flow cytometry*. If different antibodies / fluorescent dye combinations are used, the fraction of cells expressing several antigens can be determined.

11. Anti-idiotype antibodies, which are antibodies against the binding domain of another antibody, can sometimes be used to mimic the structure of the original antigen.

www.users.med.cornell.edu.

MARKETED PREPARATIONS :

(W Wang 2008)

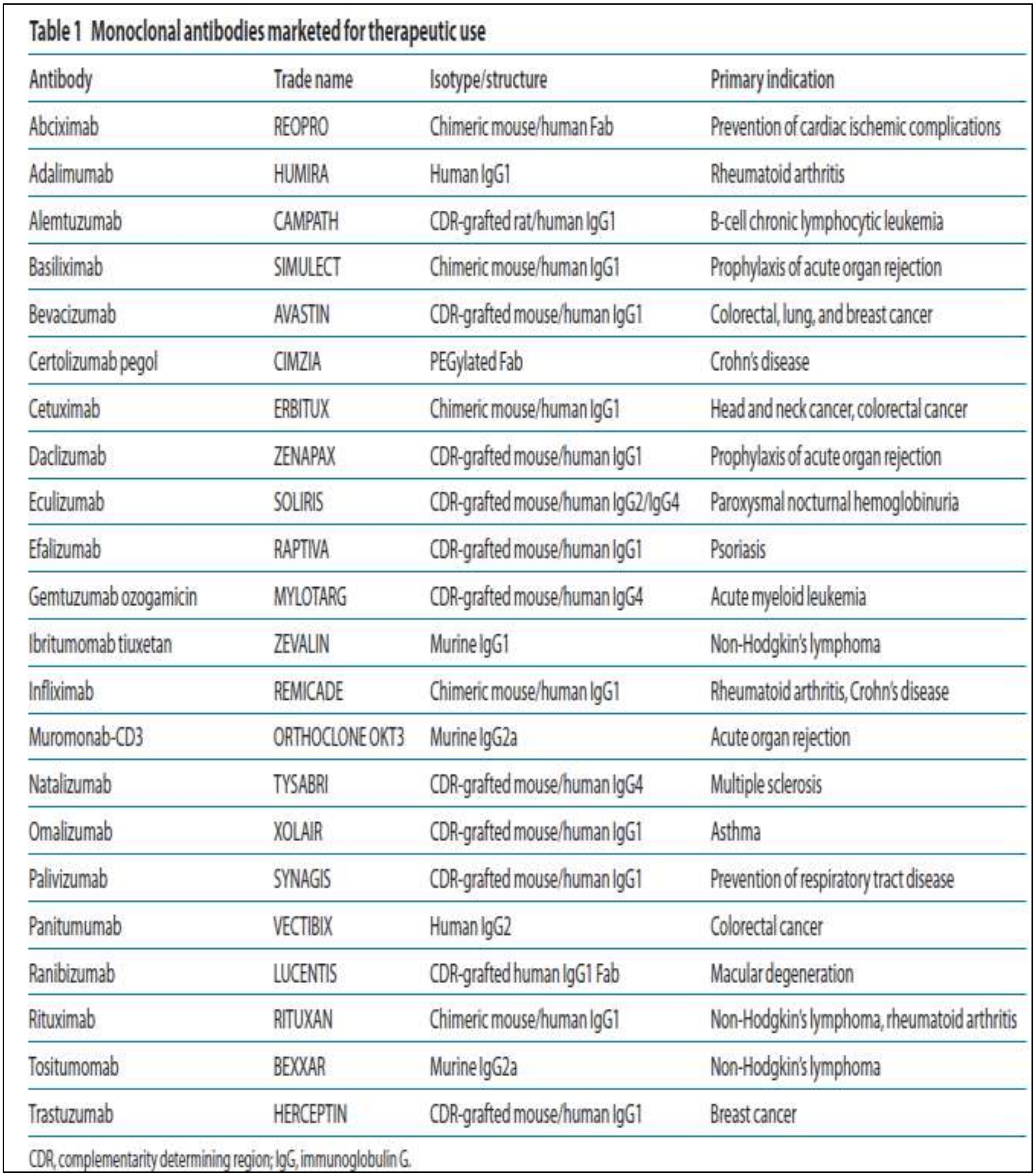

Table 1 Monoclonal antibodies marketed for therapeutic use

Antibody	Trade name	Isotype/structure	Primary indication
Abciximab	REOPRO	Chimeric mouse/human Fab	Prevention of cardiac ischemic complications
Adalimumab	HUMIRA	Human IgG1	Rheumatoid arthritis
Alemtuzumab	CAMPATH	CDR-grafted rat/human IgG1	B-cell chronic lymphocytic leukemia
Basiliximab	SIMULECT	Chimeric mouse/human IgG1	Prophylaxis of acute organ rejection
Bevacizumab	AVASTIN	CDR-grafted mouse/human IgG1	Colorectal, lung, and breast cancer
Certolizumab pegol	CIMZIA	PEGylated Fab	Crohn's disease
Cetuximab	ERBITUX	Chimeric mouse/human IgG1	Head and neck cancer, colorectal cancer
Daclizumab	ZENAPAX	CDR-grafted mouse/human IgG1	Prophylaxis of acute organ rejection
Eculizumab	SOLIRIS	CDR-grafted mouse/human IgG2/IgG4	Paroxysmal nocturnal hemoglobinuria
Efalizumab	RAPTIVA	CDR-grafted mouse/human IgG1	Psoriasis
Gemtuzumab ozogamicin	MYLOTARG	CDR-grafted mouse/human IgG4	Acute myeloid leukemia
Ibritumomab tiuxetan	ZEVALIN	Murine IgG1	Non-Hodgkin's lymphoma
Infliximab	REMICADE	Chimeric mouse/human IgG1	Rheumatoid arthritis, Crohn's disease
Muromonab-CD3	ORTHOCLONE OKT3	Murine IgG2a	Acute organ rejection
Natalizumab	TYSABRI	CDR-grafted mouse/human IgG4	Multiple sclerosis
Omalizumab	XOLAIR	CDR-grafted mouse/human IgG1	Asthma
Palivizumab	SYNAGIS	CDR-grafted mouse/human IgG1	Prevention of respiratory tract disease
Panitumumab	VECTIBIX	Human IgG2	Colorectal cancer
Ranibizumab	LUCENTIS	CDR-grafted human IgG1 Fab	Macular degeneration
Rituximab	RITUXAN	Chimeric mouse/human IgG1	Non-Hodgkin's lymphoma, rheumatoid arthritis
Tositumomab	BEXXAR	Murine IgG2a	Non-Hodgkin's lymphoma
Trastuzumab	HERCEPTIN	CDR-grafted mouse/human IgG1	Breast cancer

CDR, complementarity determining region; IgG, immunoglobulin G.

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INTRANASAL DRUG DELIVERY SYSTEMS

Wayal Abhijit, Katedeshmukh Ramesh

1. Introduction:

In the past decade, the use of the nasal cavity as a route for drug delivery has been an area of great interest to the pharmaceutical industry, especially for systemically acting drugs that are difficult to deliver via routes other than injection. The nasal route could be important for drugs that are used in crisis treatments, such as for pain, and for centrally acting drugs where the putative pathway from nose to brain might provide a faster and more specific therapeutic effect (Illum L. 2003).

The world market has seen an increasing number of systemically acting drugs being marketed as nasal formulations. A range of companies specializing in the development of innovative nasal delivery systems and formulation are actively developing novel nasal formulations for conventional generic drugs (e.g. apomorphine, triptans, morphine, midazolam, fentanyl, non-steroid anti-inflammatory drugs), as well as for peptides and proteins (e.g. leuprolide, parathyroid hormone, insulin, interferon) in situations where the nasal route would be beneficial for the therapeutic efficacy of the drug (Illum L. 2001) (Davis S. 2001)

Furthermore, the use of the nasal cavity for vaccination, especially against respiratory infections, is being pursued by vaccine companies. This is because it is possible to obtain, by the nasal route, not only a systemic immune response, but also a local mucosal immune response that should provide a much higher level of protection against these diseases This chapter will discuss new developments and strategies for nasal drug delivery and also touch upon the physicochemical and physiological factors to be considered when entering into the development of novel nasal formulations (Illum L. 2001) (Davis S. 2001).

1.1 **Advantages:** (Jadhav K.R. 2007)

1) Easy accessibility and needle free drug application without the necessity of trained personnel facilitates self medication, thus improving patient compliances compared to parenteral routes (Pontiroli A. 1991).
2) Good penetration of, especially lipophilic, low molecular weight drugs through the nasal mucosa.

For instance the absolute nasal bioavailability of fentanyl is about 80% (Striebel H.W. 1993).
3) Rapid absorption and fast onset of action due to relatively large absorption surface and high vascularization. Thus the Tmax of fentanyl after nasal administration was less than or equal to 7 minute comparable to intravenous [i.v] (Striebel H.W. 1993). Nasal administration of suitable drug would therefore be effective in emergency therapy as an alternative to parenteral administration routes.
4) Avoidance of the harsh environmental conditions in the gastrointestinal tract (chemical and enzymatic degradation of drugs).
5) Avoidance of hepatic first pass metabolism and thus potential for dose reduction compared to oral delivery.
6) Potential for direct delivery of drug to the central nervous system via the olfactory region, thus by-passing the blood brain barrier (Talegaonkar S. 2004).
7) Direct delivery of vaccine to lymphatic tissue and induction of a secretory immune response at distant mucosal site (Davis S. 2001).

1.2 **Limitations:** (Hirai S. 1981) (Kadam S.S. 1993)

1) The histological toxicity of absorption enhancers used in nasal drug delivery system is not yet clearly established.
2) Relatively inconvenient to patients when compared to oral delivery systems since there is a possibility of nasal irritation.
3) Nasal cavity provides smaller absorption surface area when compared to GIT.
4) There is a risk of local side effects and irreversible damage of the cilia on the nasal mucosa, both from the substance and from constituents added to the dosage form.

5) Certain surfactants used as chemical enhancers may disrupt and even dissolve membrane in high concentration.
6) There could be a mechanical loss of the dosage form into the other parts of the respiratory tract like lungs because of the improper technique of administration (Aurora J. 2002).

2. **Nasal anatomy:**

The nose is divided into two nasal cavities *via* the septum. The volume of each cavity is approximately 7.5mL and has a surface area around 75 cm^2. There are three distinct functional regions in the nose- the vestibular, respiratory, and olfactory. Among these, the respiratory region is the most important for systemic drug delivery (Jadhav K.R. 2007).

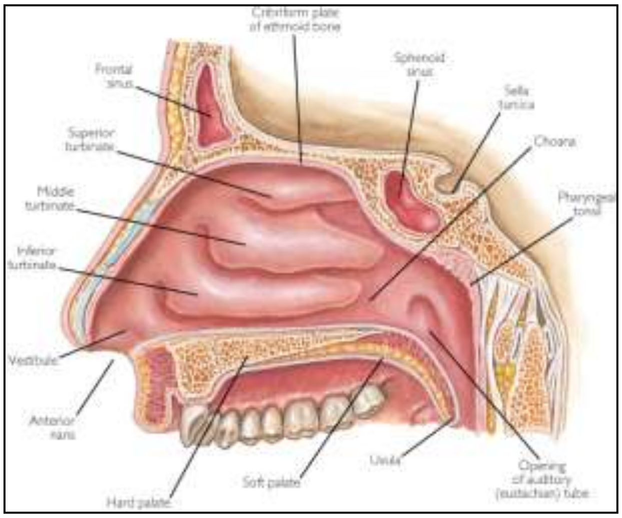

Fig.1 Nasal anatomy

The vestibular region: It is located at the opening of nasal passages and is responsible for filtering out the air borne particles. It is considered to be the least important of the three regions with regard to drug absorption.

The respiratory region: The respiratory region is the largest having the highest degree of vascularity and is mainly responsible for systemic drug absorption.

The olfactory region: It is of about 10 cm^2 in surface area, and it plays a vital role in transportation of drugs to the brain and the CSF. Human olfactory region comprises of thick connective tissue lamina propria, upon which rests the olfactory epithelium. Lamina propria has axons, bowans bundle and blood vessels whereas epithelium consists of three different cells i.e. basal cells, supporting cells and olfactory receptor cells. Neurons are interspersed between supporting cells.
The epithelium of the nasal passage is covered by a mucus layer, which entraps particles. The mucus layer is cleared from the nasal cavity by cilia, and is renewed every 10 to 15 minutes. The pH of the mucosal secretions ranges from 5.5 to 6.5 in adults and 5.0 to 6.7 in children. The mucus moves through the nose at an approximate rate of 5 to 6 mm/min resulting in particle clearance within the nose every 15 to 20 minutes. Numerous enzymes for instance, cytochrome P450 enzymes, carboxylesterases and glutathione S-transferases are found in nasal cavity (Dhakar R.C. 2011).

3. **Mechanism of drug absorption:**

The first step in the absorption of drug from the nasal cavity is passage through the mucus. Small, unchanged particles easily pass through this layer. However, large or charged particles may find it more difficult to cross. Mucin, the principle protein in the mucus, has the potential to bind to solutes, hindering diffusion. Additionally, structural changes in the mucus layer are possible as a result of environmental changes (i.e. pH, temperature, etc.). Subsequent to a drug's passage through the mucus, there are several mechanisms for absorption through the mucosa. These include transcellular or simple diffusion across the membrane, paracellular transport via movement between cell and transcytosis by vesicle carriers. Several mechanisms have been proposed but the following two mechanisms have been considered predominantly.

3.1 First mechanism

It involves an aqueous route of transport, which is also known as the paracellular route but slow and passive. There is an inverse log-log correlation between intranasal absorption and the molecular weight of water-soluble compounds. The drugs having molecular weight greater than 1000 Daltons show poor bioavailability (Aurora J. 2002).

3.2 Second mechanism

It involves transport through a lipoidal route and it is also known as the transcellular process. It is responsible for the transport of lipophilic drugs that show a rate dependency on their lipophilicity. Drugs also cross cell membranes by an active transport route via carrier-mediated means or transport through the opening of tight junctions (Aurora J. 2002).

4. **Barriers to nasal absorption:** (Jadhav K.R. 2007)

4.1 Low bioavailability

Bioavailability of polar drugs is generally low, about 10% for low molecular weight drugs and not above 1% for peptides such as calcitonin and insulin (Illum L. 2002). The most important factor limiting the nasal absorption of polar drugs and especially large molecular weight polar drugs such as peptides and proteins is the low membrane permeability. Drugs can cross the epithelial cell membrane either by the transcellular route exploiting simple concentration gradients, by receptor mediated or vesicular transport mechanisms, or by the paracellular route through the tight junctions between the cells. Polar drugs with molecular weights below 1000 Da will generally pass the membrane using the latter route. Although tight junctions are dynamic structures and can open and close to a certain degree when needed, the mean size of these channels is of the order of less than 10 Å and the transport of larger molecules is considerably more limited (McMartin C. 1987). Larger peptides and proteins are able to pass the nasal membrane using an endocytotic transport process but only in low amounts. Nasal absorption of such polar drugs can be greatly improved by co-administration of absorption enhancing agents.

1.	Nasal enzyme inhibitors	Bestatin, amastatin, borileucine, fusidic acid, bile salts
2.	Nasal permeation enhancers	Cyclodextrins, surfactants, saponins, phospholipids
3.	Prodrug approach	Cyclic prodrug, esters, derivatization of of C and N termini
4.	Nasal mucoadhesives in nasal drug delivery	Carbopol, polycarbophil, cellulose derivatives, chitosan, lecithin
5.	Particulate drug delivery	Nanospheres, microspheres, liposomes

Table.1 Strategies to improve nasal absorption (Upadhyay S. 2011)

4.2 Mucociliary clearance

The general fast clearance of the administered formulation from the nasal cavity due to the mucociliary clearance mechanism is another factor of importance for low membrane transport. This is especially the case when the drug is not absorbed rapidly enough across the nasal mucosa. It has been shown that for both liquid and powder formulations, which are not bioadhesive, the half life for clearance is of the order of 15 - 30 min. The use of bioadhesive excipients in the formulations is an approach to overcome the rapid mucociliary clearance (Kublik H. 1998) (Harris A.S. 1986)

4.3 Enzymatic degradation

Another contributing, but often less considered factor to the low bioavailability of peptides and proteins across the nasal mucosa is the possibility of an enzymatic degradation of the molecule in the lumen of the nasal cavity or during passage through the epithelial barrier. These sites both contain exopeptidases such as mono and diaminopeptidases that can cleave peptides at their N and C termini and endopeptidases such as serine and cysteine, which can attack internal peptide bonds. The use of enzyme inhibitors and/or saturation of enzymes may be approaches to overcome this barrier (Lee V.H.L. 1988).

5. **The Blood Brain Barrier and nasal drug delivery for targeting brain:**

Targeting of drugs to the central nervous system (CNS) is still a difficult task to fulfill because the blood–brain barrier (BBB), segregating the brain interstitial fluid from the circulating blood, and the blood–cerebrospinal fluid barrier, separating the blood from the Cerebrospinal fluid, prevents the influx of hydrophilic compounds with a molecular weight above 600 Da from the systemic circulation into the brain. Hence, these barriers actively controlling cellular and molecular trafficking prevent the utilization of many novel therapeutic agents for treating CNS disorders such as Parkinson’s and Alzheimer’s diseases (Horvát S. 2009).

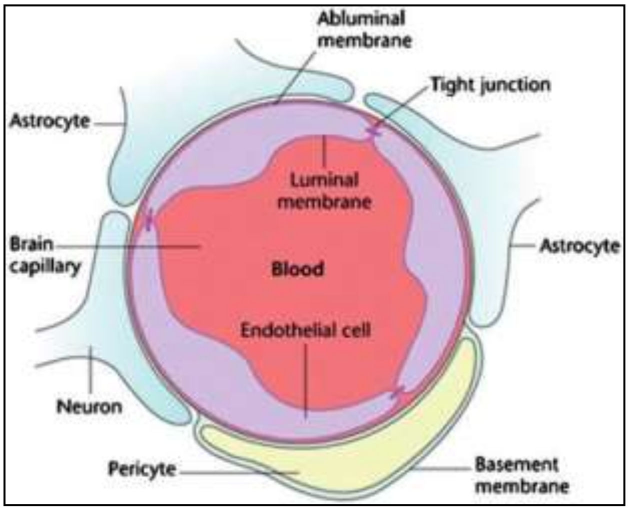

Fig.2 Blood Brain Barrier

One technique that holds promise for bypassing the BBB to deliver drugs to the brain and eliminating the surgical risk and the spillover effect of drug to normal tissue is intranasal delivery. Intranasal delivery provides a practical, noninvasive method for delivering therapeutic agents to the brain because of the unique anatomic connections provided by the olfactory and trigeminal nerves. These nerves connect the nasal mucosa and central nervous system (CNS), allowing them to detect odors and other chemical stimuli.

Intranasally administered drugs reach the brain parenchyma, spinal cord, and cerebrospinal fluid (CSF) within minutes by using an extracellular route through perineural and/or perivascular channels along the olfactory and trigeminal nerves without binding to any receptor or using axonal transport. In addition to bypassing the BBB, advantages of intranasal delivery include rapid delivery to the CNS, avoidance of hepatic first-pass drug metabolism, and elimination of the need for systemic delivery, thereby reducing unwanted systemic side effects.

Many therapeutic agents, including growth factors, proteins, peptides, viral vectors, liposomes, and vaccines, have been delivered to the CNS through the nasal route and applied for the treatment of CNS disorders in both animals and humans.

It has been reported that insulin-like growth factor-1 can be rapidly transported into the rat brain and upper spinal cord via the olfactory and trigeminal pathways. In humans, intranasal delivery of insulin has been shown to improve memory in normal adults and in patients with early Alzheimer’s disease without changing blood levels of glucose or insulin. Also, intranasal oxytocin has been reported to improve trust in humans.

In brain tumors, anticancer agents such as methotrexate, 5-fluorouracil, and raltitrexed have been delivered successfully to the brain using intranasal delivery (Hashizume R. 2008).

5.1 Factors affecting nasal drug delivery to the brain

The size of the molecule is the major determinant in whether a substance will be absorbed across the nasal respiratory epithelium and/or transported along the olfactory pathway. Fisher et al. demonstrated an almost linear relationship between the log (molecular weight) and the log (% drug absorbed) of water-soluble compounds.

Other factors affecting delivery to the brain include the degree of dissociations and lipophilicity (higher lipophilicity results in better transport). Once a drug is in the brain, it can be further influenced by BBB efflux transporter systems like P-glycoprotein (P-gp). However, it was found that uptake into the brain was enhanced when drugs were administered in combination with the P-gp efflux inhibitor, rifampin.

6. **Reason for development of nasal delivery:**

Nasal drug delivery is a useful delivery method for drugs that are active in low doses and show no or minimal oral bioavailability. The nasal route circumvents hepatic first pass elimination associated with the oral delivery: it is easily accessible and suitable for self-medication. Currently, tow classes of nasally delivered therapeutics are on the market. The first one comprises low molecular weight and hydrophobic drugs for the treatment of the nasal mucosa and sinus, including decongestants, topical steroids, antibiotics and other (OTC) products. The second class encompasses a few drugs, which have sufficient nasal absorption for displaying systemic effects. Important candidates are the compounds, generally administered by injection and hardly absorbed after oral administration, due to their instability in gastrointestinal tract, poor absorption properties, and their rapid and extensive biotransformation (Dhakar R.C. 2011).

7. **Factors affecting the characteristics of nasal drug delivery:** (Dhakar R.C. 2011)

Some of the physicochemical, formulation and physiological factors are imperative and must be considered prior to designing intranasal delivery.

7.1 Physicochemical properties of drugs

Chemical form: The chemical form of a drugis important in determining absorption.
For example, conversion of the drug into a salt or ester form can also alter its absorption. Huang et al 1985 studied the effect of structural modification of drug on absorption (Huang C. 1985). It was observed that in-situ nasal absorption of carboxylic acid esters of L-Tyrosine was significantly greater than that of L-Tyrosine.

Polymorphism: Polymorphism is known to affect the dissolution rate and solubility of drugs and thus their absorption through biological membranes.

Molecular Weight: A linear inverse correlation has been reported between the absorption of drugs and molecular weight up to 300 Da. Absorption decreases significantly if the molecular weight is greater than 1000 Da except with the use of absorption enhancers. Shape is also important. Linear molecules have lower absorption than cyclic – shaped molecules.

Particle Size: It has been reported that particle sizes greater than 10μm are deposited in the nasal cavity.
Solubility & dissolution Rate: Drug solubility and dissolution rates are important factors in determining nasal absorption from powders and suspensions. The particles deposited in the nasal cavity need to be dissolved prior to absorption. If a drug remains as particles or is cleared away, no absorption occurs.

7.2 Formulation factors

i) pH of the formulation: Both the pH of the nasal cavity and pKa of a particular drug need to be considered to optimize systemic absorption. Nasal irritation is minimized when products are delivered with a pH range of 4.5 to 6.5. Also, volume and concentration are important to consider. The delivery volume is limited by the size of the nasal cavity. An upper limit of 25 mg/dose and a volume of 25 to 200μL/ nostril have been suggested for the following reasons:

- To avoid irritation of nasal mucosa
- To allow the drug to be available in unionized form for absorption
- To prevent growth of pathogenic bacteria in the nasal passage
- To maintain functionality of excipients such as preservatives
- To sustain normal physiological ciliary movement

ii) Buffer capacity: Nasal formulations are generally administered in small volumes ranging from 25 to 200μL. Hence, nasal secretions may alter the pH of the administrated dose. This can affects the concentration of unionized drug available for absorption. Therefore, an adequate formulation buffer capacity may be required to maintain the pH in-situ.

iii) Osmolarity: Drug absorption can be affected by tonicity of formulation. Shrinkage of epithelial cells has been observed in the presence of hypertonic solutions. Hypertonic saline solutions also inhibit or cease ciliary activity. Low pH has a similar effect as that of a hypertonic solution.

iv) Gelling / Viscosity building agents or gel-forming carriers: It has been studied that increase in solution viscosity may provide a means of prolonging the therapeutic effect of nasal preparations (Pennington A.K. 1988). A drug carrier such as hydroxypropyl cellulose is effective for improving the absorption of low molecular weight drugs but does not produce the same effect for high molecular weight peptides (Suzuki Y. 1999).

v) Solubilizers: Aqueous solubility of drug is always a limitation for nasal drug delivery in solution. Conventional solvents or co-solvents such as glycols, small quantities of alcohol, Transcutol (diethylene glycol monoethyl ether), medium chain glycerides and Labrasol can be used to enhance the solubility of drugs. Other options include the use of surfactants or cyclodextrins such as HP-β-cyclodextrin that serve as a biocompatible solubilizer and stabilizer in combination with lipophilic absorption enhancers.

vii) Preservatives: Most nasal formulations are aqueous based and need preservatives to prevent microbial growth. Parabens, benzalkonium chloride, phenyl ethyl alcohol, EDTA and benzoyl alcohol are some of the commonly used preservatives in nasal formulations. It has been shown that mercury containing preservatives have a fast and irreversible effect on ciliary movement and should not be used in the nasal systems (Van De Donk H.J.M. 1980).

vii) Antioxidants: Usually, antioxidants do not affect drug absorption or cause nasal irritation. Commonly used antioxidants are sodium metabisulfite, sodium bisulfite, butylated hydroxyl toluene and tocopherol.

viii) Humectants: Many allergic and chronic diseases are often connected with crusts and drying of mucous membrane. Therefore humectants can be added especially in gel-based nasal products. Humectants avoid nasal irritation and are not likely to affect drug absorption. Common examples include glycerin, sorbitol and mannitol.

ix) Role of absorption enhancers: Absorption enhancers may be required when a drug exhibits poor membrane permeability, large molecular size, lack of lipophilicity and enzymatic degradation by amino peptidases. Osmolarity and pH may accelerate the enhancing effect. Absorption enhancers improve absorption through many different mechanisms, such as increasing membrane fluidity, increasing nasal blood flow, decreasing mucus viscosity, and enzyme inhibition.

8. **Various nasal formulations:**

Recently, nasal delivery systems have been investigated with the aim of altering the pharmacokinetics of orally and parenterally administered drugs in a fashion that can enhance their pharmacologic profiles. In fact, the large surface area, porous endothelial basement membrane and high total blood flow of the nasal mucosa ensure systemic availability of compounds under circumvention of the hepatic first-pass metabolism. Nasal pharmaceutical formulations able to provide immediate drug permeation across nasal mucosa and a rapid onset of action can be good candidate particularly for patients who may have difficulty swallowing tablets or for patients who refuse intramuscular therapy.

The various formulations used in nasal drug delivery are as follows (Upadhyay S. 2011):

8.1 Liquid nasal formulations

Liquid preparations are the most widely used dosage forms for nasal administration of drugs. They are mainly based on aqueous state formulations. Their humidifying effect is convenient and useful, since many allergic and chronic diseases are often connected with crusts and drying of mucous membranes. Microbiological stability, irritation and allergic rhinitis are the major drawbacks associated with the water-based dosage forms because the required preservatives impair mucociliary function (Zia H. 1993).

i) Instillation and rhinyle catheter
Catheters are used to deliver the drops to a specified region of nasal cavity easily. Place the formulation in

the tube and kept tube one end was positioned in the nose, and the solution was delivered into the nasal cavity by blowing through the other end by mouth (Harris A.S. 1986).

ii) Compressed air nebulizers

Nebulizer is a device used to administer medication in the form of a mist inhaled into the lungs. The compressed air is filled into the device, so it is called compressed air nebulizers. The common technical principle for all nebulizers, is to either use oxygen, compressed air or ultrasonic power, as means to break up medical solutions/ suspensions into small aerosol droplets, for direct inhalation from the mouthpiece of the device (Knoch M. 2002).

iii) Squeezed bottle

Squeezed nasal bottles are mainly used as delivery device for decongestants. They include a smooth plastic bottle with a simple jet outlet. While pressing the plastic bottle the air inside the container is pressed out of the small nozzle, thereby atomizing a certain volume. By releasing the pressure again air is drawn inside the bottle. This procedure often results in contamination of the liquid by microorganisms and nasal secretion sucked inside (Mygind N. 1978).

iv) Metered-dose pump sprays

Most of the pharmaceutical nasal preparations on the market containing solutions, emulsions or suspensions are delivered by metered-dose pump sprays. Nasal sprays, or nasal mists, are used for the nasal delivery of a drug or drugs, either locally to generally alleviate cold or allergy symptoms such as nasal congestion or systemically, see nasal administration. Although delivery methods vary, most nasal sprays function by instilling a fine mist into the nostril by action of a hand-operated pump mechanism. The three main types available for local effect are: antihistamines, corticosteroids, and topical decongestants

8.2 Powder dosage Forms

Dry powders are less frequently used in nasal drug delivery. Major advantages of this dosage form are the lack of preservatives and the improved stability of the formulation. Compared to solutions, the administration of powders could result in a prolonged contact with the nasal mucosa.

i) Insufflators

Insufflators are the devices to deliver the drug substance for inhalation; it can be constructed by using a straw or tube which contains the drug substance and sometimes it contains syringe also. The achieved particle size of these systems is often increased. compared to the particle size of the powder particles due to insufficient deaggregation of the particles and results in a high coefficient of variation for initial deposition areas. Many insufflator systems work with pre-dosed powder doses in capsules (Hughes B.L. 1993).

ii) Dry powder inhaler

Dry powder inhalers (DPIs) are devices through which a dry powder formulation of an active drug is delivered for local or systemic effect via the pulmonary route. Dry powder inhalers are bolus drug delivery devices that contain solid drug, suspended or dissolved in a non polar volatile propellant or in dry powder inhaler that is fluidized when the patient inhales (Alagusundaram M. 2010).

8.3 Pressurized MDIs

A metered-dose inhaler (MDI) is a device that delivers a specific amount of medication in the form of a short burst of aerosolized medicine that is inhaled by the patient. The advantages of MDIs are their portability and small size, availability over a wide dosage range per actuation, dose consistency, dose accu-racy, protection of the contents and that they are quickly ready for use.

Propellants in MDIs typically make up more than 99 % of the delivered dose. Actuation of the device releases a single metered dose of the formulation which contains the medication either dissolved or suspended in the propellant. Breakup of the volatile propellant into droplets, followed by rapid evaporation of these droplets, results in the generation of an aerosol consisting of micrometer sized medication particles that are then inhaled.

8.4 Nasal gels

Nasal gels are high viscosity thickened solutions or suspensions. Until the recent development of precise dosing devices, there was not much interest in this system. The advantages of a nasal gel include the reduction of post nasal drip due to high viscosity, reduction of taste impact due to reduced swallowing, reduction of anterior leakage of the formulation, reduction of irritation by using soothing/emollient excipients and target delivery to mucosa for better absorption. Without special application techniques it only occupies a narrow distribution area in the nasal cavity, where it is placed directly. Recently, the first nasal gel containing Vitamin B12 for systemic medication has entered the market (Junginger H.E. 1956).

9. **Evaluation of nasal formulations:**

9.1 In vitro nasal permeation studies

Various approaches used to determine the drug diffusion through nasal mucosa from the formulation. The two important methodologies to study the diffusion profile of the drug are discussed here,

In vitro diffusion studies

The nasal diffusion cell is fabricated in glass. The water-jacketed recipient chamber has total capacity of 60 ml and a flanged top of about 3mm; the lid has 3 opening, each for sampling, thermometer, and a donor tube chamber. The 10 cm long donor chamber, and a donor tube chamber has total capacity of 60 ml and a flanged top of about 3mm; the lid has 3 openings, each for sampling, thermometer, and a donor tube chamber the 10 cm long donor chamber tube has internal diameter of 1.13 cm. The nasal mucosa of sheep was separated from sub layer bony tissues and stoned in distilled water containing few drops at genatamycin injection. After the complete removal of blood from muscosal surface, is attached to donor chamber tube. The donor chamber tube is placed such a way that it just touches the diffusion medium in recipient chamber. At predetermined intervals, samples (0.5 ml) from recipient chamber are with draw and transferred to amber colored ampoules. The samples withdrawn are suitably replaced. The samples are estimated for drug content by suitable analytical technique. Through out the experiment the temperature is maintained at 37^{o}C.

9.2 In Vivo Nasal Absorption studies

Animal models for nasal absorption studies

The animal models employed for nasal absorption studies can be of two types, viz., whole animal or *in vivo* model and an isolated organ perfusion or *ex vivo* model. These models are discussed in detail below:

Rat Model

The surgical preparation of rat for *in vivo* nasal absorption study is carried out as follows: The rat is anaesthetized by intraperitoneal injection of sodium pentobarbital. An incision is made in the neck and the trachea is cannulated with a polyethylene tube. Another tube is inserted through the oesophagus towards the posterior region of the nasal cavity. The passage of the nasopalatine tract is sealed so that the drug solution is not drained from the nasal cavity through the mouth. The drug solution is delivered to the nasal cavity through the nostril or through the cannulation tubing. The blood samples are collected from the femoral vein. As all the probable outlets of drainage are blocked, the drug can be only absorbed and transported into the systemic circulation by penetration and/or diffusion through nasal mucosa.

Rabbit Model

The rabbit offers several advantages as an animal model for nasal absorption studies:

1. It is relatively cheap, readily available and easily maintained in laboratory settings
2. It permits pharmacokinetic studies as with large animals (like monkey)
3. The blood volume is large enough (approx. 300ml)
4. To allow frequent blood sampling (l-2ml)

Thus it permits full characterization of the absorption and determination of the pharmacokinetic profile of a drug. Rabbits (approx. 3 kg) are either anaesthetized or maintained in the conscious state depending on the purpose of study. In the anaesthetized model, the rabbit is anaesthetized by an intramuscular injection of a combination of ketamine and xylazine. The rabbit's head is held in an upright position and the drug solution is administered by nasal spray into each nostril. During the experiment the body temperature of the rabbit is maintained at 37°C with the help of a heating pad. The blood samples are collected by an indwelling catheter in the marginal ear vein or artery.

Dog Model

The dog is either anaesthetized or retained hi the conscious condition depending on the drug characteristics and the purpose of experiment. The dog is anaesthetized by intravenous injection of sodium thiopental and the anesthesia is maintained with sodium Phenobarbital. A positive pressure pump through a cuffed endotracheal tube gives the ventilation. The body temperature is maintained at 37-38°C by a heating pad. The blood sampling is carried out from the jugular vein.

Sheep Model

The sheep, rabbit and dog models are more practical and suitable for investigating nasal drug delivery from sophisticated formulations. They permit better evaluation of the parameters there involved. The *in vivo* sheep model for nasal delivery is essentially parallel to that for the dog model. Male in-house bred sheep are employed since they are free from nasal infections.

9.3 Ex Vivo Nasal Perfusion Models

Surgical preparation is the same as that is for *in vivo* rat model. During the perfusion studies, a funnel is placed between the nose and reservoir to minimize the loss of drug solution. The drug solution is placed in a reservoir maintained at 37°C and is circulated through the nasal cavity of the rat with a peristaltic pump. The perfusion solution passes out from the nostrils (through the funnel) and runs again into the reservoir. The drug solution in the reservoir is continuously stirred. The amount of drug absorbed is estimated by measuring the residual drug concentration in the perfusing solution. The drug activity due to stability problems may be lost during the course of experiment. This is especially true for peptide and protein drugs that may undergo proteolysis and aggregation.

Rabbit can also be used as the animal model for *ex vivo* nasal perfusion studies. The rabbit is anaesthetized with parenteral uretliane-acepromazine. A midline incision is made in the neck and the trachea is cannulated with a polyethylene neonatal endotracheal tube. The oesophagus is isolated and ligated. The distal end of the oesophagus is closed with suture and flexible tygon tubing is inserted into the proximal end and advanced to the posterior part of the nasal cavity. The nasopalatine tract (that connects nasal cavity to the mouth) is closed with an adhesive to avoid drainage of drug solution from the nasal cavity. The drug in isotonic buffer solution is recirculated using a peristaltic pump.

10. **Application of nasal drug delivery system:** (Rahisuddin 2011)

10.1 Local delivery

For the natural treatment of topical nasal disorders the drug is administered through nasal route. Among the most common examples are antihistamines and corticosteroids for rhino sinusitis, and nasal decongestants for cold symptoms. In fact, relatively low doses are effective when administered through nasal route with less systemic toxic effects.

10.2 Systemic delivery

The intranasal administration of drugs is an effective way for systemic availability of drugs as compared to oral and intravascular routes. Actually, it seems to present fast and extended drug absorption, and it has been supported by many studies planned to compare intranasal drug delivery against oral and parenteral administration. Examples include analgesics (morphine), cardiovascular drugs as propranolol and carvedilol, hormones such as levonorgestrel, progesterone and insulin, anti-inflammatory agents as indomethacin and ketorolac, and antiviral drugs (acyclovir). Some examples which are available in the market include zolmitriptan and sumatriptan for the treatment of migraine and cluster headaches.

10.3 Nasal vaccines

Nasal mucosa is the first site of contact with inhaled antigens and therefore, its use for vaccination, especially against respiratory infections, has been extensively evaluated. In fact, nasal vaccination is a promising alternative to the classic parenteral route, because it is able to enhance the systemic levels of specific immunoglobulin G and nasal secretary immunoglobulin A. Examples of the human efficacy of intranasal vaccines include those against influenza A and B virus, proteosoma influenza, adenovirus vectored influenza, group B meningococcal native, attenuated respiratory syncytial virus and parainfluenza 3 virus.

10.4 CNS delivery through nasal route

Intranasal route has promising approaches for delivery of drugs to the brain. The delivery of drugs to the CNS from the nasal route may occur via olfactory neuroepithelium. The transport via trigeminal nerve system from the nasal cavity to CNS has also been described. Drug delivery through nasal route into CNS has been reported for Alzheimer's disease, brain tumors, epilepsy, pain and sleep disorders.

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PROTEINS AND PEPTIDE DRUG DELIVERY SYSTEM

Zarikar Nitin, Hastak Vishakha

Introduction:

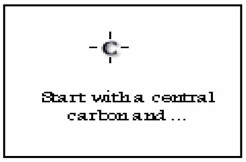
Proteins and peptides are the biopolymers which yield two or more amino acids on hydrolysis. Peptides and polypeptides are the principal components of the protoplasm of cells and are high molecular weight compounds consisting of alpha amino acids connected together by peptide linkages. (Gupta H. 2009)

Peptides are short polymers formed from the linking of (usually less than or equal to 100) amino acids and comprise some of the basic components of human biological processes, including enzymes, hormones, and antibodies. The link between one amino acid residue and the next is known as a peptide bond or an amide bond – formed when a carboxyl group reacts with an amine group of an adjacent residue – giving the chemical its name. Proteins longer chains of (greater than 100) amino acids similarly linked by peptide bonds. There are various distinctions between peptides and proteins, but to define them simply: peptide chains are short and proteins long. Protein and peptides are available in various dosage forms like solutions, emulsion, suspension, dry powder, sprays etc… Till recent, injections remained the most common means for administering therapeutic proteins and peptides because of their poor oral bioavailability. However, oral route would be preferred to any other route because of its high levels of patient acceptance and long term compliance, which increases the therapeutic value of the drug. Designing and formulating a polypeptide drug delivery through the gastro intestinal tract has been a persistent challenge because of their unfavorable physicochemical properties, which includes enzymatic degradation, poor membrane permeability and large molecular size. The main challenge is to improve the oral bioavailability from less than 1% to at least 30-50%. Consequently, efforts have intensified over the past few decades, where every oral dosage form used for the conventional small molecule drugs has been used to explore oral protein and peptide delivery. Various strategies currently under investigation include chemical modification, formulation vehicles and use of enzyme inhibitors, absorption enhancers and mucoadhesive polymers. Structure of protein and peptides. (GARY H. n.d.)

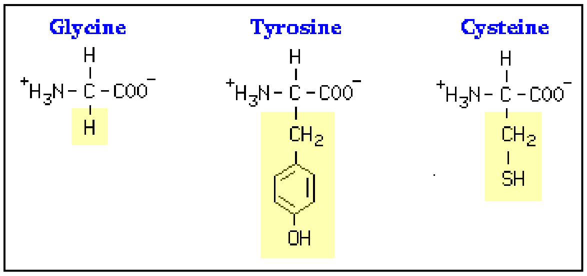
Amino Acids:

Proteins are polymers of amino acids joined together by peptide bonds. There are 20 different amino acids that make up essentially all proteins on earth. Each of these amino acids has a fundamental design composed of a central carbon (also called the alpha carbon) bonded to:

- a hydrogen
- a carboxyl group
- an amino group
- a unique side chain or R-group



Thus, the characteristic that distinguishes one amino acid from another is its unique side chain, and it is the side chain that dictates an amino acids chemical properties.



The unique side chains confer unique chemical properties on amino acids, and dictate how each amino acid interacts with the others in a protein. Amino acids are covalently bonded together in chains by peptide bonds. If the chain length is short (say less than 100 amino acids) it is called a peptide; longer chains are called polypeptides or proteins. Peptide bonds are formed between the carboxyl group of one amino acid and the amino group of the next amino acid. Peptide bond formation occurs in a condensation reaction involving loss of a molecule of water.

Structural features of proteins are usually described at four levels of complexity:

- **Primary structure:** the linear arrangement of amino acids in a protein and the location of covalent linkages such as disulfide bonds between amino acids.
- **Secondary structure:** areas of folding or coiling within a protein; examples include alpha helices and pleated sheets, which are stabilized by hydrogen bonding
- **Tertiary structure:** the final three-dimensional structure of a protein, which results from a large number of non-covalent interactions between amino acids.
- **Quaternary structure:** non-covalent interactions that bind multiple polypeptides into a single, larger protein. Hemoglobin has quaternary structure due to association of two alpha globin and two beta globin polyproteins. (Review of Nucleic Acid and Protein Chemistry 2002)

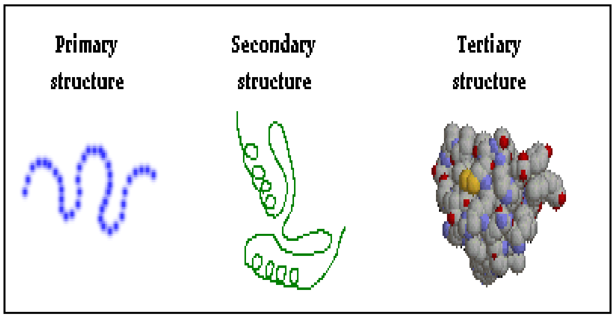


Fig: 1. Structure of protein.

2) Need for protein and peptide drug delivery system

The role of protein and peptides in the biological system is immense. Lack of a specific protein due to the diseased state of the secreting organ can disrupt the normal functions of the biologic system, which can at a particular time prove to be fatal. Diabetes mellitus is the result of absolute or the relative deficiency of the insulin in the body. Insulin is a protein secreted from the pancreas and lack of insulin leads to eventual death if insulin is not supplied by exogenous source.

In fact, protein instability is one of the main reasons why protein pharmaceuticals are administered traditionally through injections rather than taken orally like most chemical drugs. Although protein and peptides on oral administration are capable of traversing through the intestinal epithelium in the intact form, pre-systemic degradation in the gastrointestinal track, result in poor bioavaibility. As a result of this, the commonly used method of systemic administration of protein and peptides drugs is by the parenteral route.

Although one of the simplest form of parenteral administration of protein /peptides is direct injection into the blood stream, here one must consider properties of polypeptides that may impede their delivery, including

a) Chemical, enzymatic physical instability.

b) Their potential for provoking an immunogenic response.

c) Their inability to be transported intact form the vascular compartment to vascular site(s) of action with efficiency suitable to support therapeutic needs. (Jain N.K. 2005)

3) Challenges in protein and peptide drug delivery

1. Proteins are relatively large molecules with complex architecture

2. Disruption of protein structure (Primary, Secondary, Tertiary, Quaternary) by presence of hydrophobic surface, removal of water and change in temperature or pH or modification of the side chains, can lead to loss of activity or immunogenicity.

3. Molecular sizes, short plasma half life, requirement of specialized mechanisms for transport also contribute towards problems associated with the delivery of proteins and peptides.

4. There are the usual challenges inherent in protein/peptide formulation development, such as maintaining physicochemical stability as well as the biological activity of the API throughout the product shelf-life, and ensuring efficient utilization of the often costly protein or peptide API throughout both the manufacturing and the delivery process. (Jain N.K 2005)

Manufacturing Techniques:

Peptides:

Peptides are manufactured utilizing two distinct techniques:

1. Solution phase.

2. Solid phase.

Each has unique applications and their implementation can greatly affect the cost and scalability of the pharmaceuticals that incorporate their, respective peptides. Liquid- or solution-based peptide synthesis is the older of the techniques with most labs using solid phase synthesis today. The method is better for shorter peptide chains and is still useful in large-scale production greater than 100 kg in scale. Solid phase synthesis allows for an innate mixing of natural peptides that are difficult to express in bacteria. It can incorporate amino acids that do not occur naturally and modify the peptide/protein backbone. In this method, amino acids attach to polymer beads suspended in a solution to build peptides. They remain attached to beads until cleaved by a reagent such as trifluoroacetic acid. This immobilizes the peptide during the synthesis so it can be captured during filtration. Liquid phase reagents and by-products are simply flushed away. The benefits of solid phase synthesis include: higher speed of peptide production, as it is a relatively simple process, and easy scale-up. It is also more suitable than solution phase synthesis for longer sequences.

Within solid phase there are two different methods:

1) t-Boc [(t)ert-(B)ut(o)xy(c)arbonyl]

2) Fmoc [9H-(f)luoren-9-yl(m)eth(o)xy(c)arbonyl]

T-Boc is the original method used in solid phase synthesis. It uses an acidic condition to remove Boc from a growing peptide chain. The method requires the use of small quantities of hydrofluoric acid which is generally regarded as safe for specialized equipment. This method is preferred for complex syntheses and when synthesizing non-natural peptides. Fmoc, pioneered later than t-Boc, makes cleaving peptides uncomplicated. It is also easier to hydrolyze the peptide from the resin with a weaker acid. This eliminates the need for specialized equipment. Again, both methods are valuable and each suits specific applications. However, Fmoc is more widely used because it eliminates the need for hydrofluoric acid.

Proteins:

Manufacturing biotech drugs is a complicated and time consuming process, and it can take many years just to identify the therapeutic protein, determine its gene sequence, and validate a process to make the molecules using biotechnology. Prior to advances in biotechnology such as recombinant DNA (rDNA) and hybridoma cell technology, the few protein drugs available were derived from human and animal corpses. In fact, the human growth hormone was taken from human corpses, and the insulin required to treat diabetes was collected from slaughtered pigs. Given their source, these drugs were expensive and available in limited supplies. Hybridoma cell and rDNA technologies, however, have provided a cost-effective way to produce protein-based drugs in bulk quantities. Hybridomas are the fusion of tumor cells with certain white blood cells. This fusion causes endless replication for use in the production of specific protein-based drugs called monoclonal antibodies which are effective in treating cancers and other ailments. The introduction of rDNA technology, or genetic engineering, has allowed the gene that encodes for the required protein to be transferred from one organism into another, enabling larger amounts of the drug to be produced. As part of the process, host cells that have been transformed to contain the gene of interest are grown in carefully controlled conditions in large stainless-steel tanks. The cells are then stimulated to produce the target proteins through very specific culture conditions, including maintaining a suitable balance of temperature, oxygen, and acidity, among other variables. After careful culture, the proteins are isolated from the cultures and put through a rigorous test at every step of purification before being formulated into pharmaceutically active products. This complex process is bound by the FDA Guidance for Industry document: “Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice” which includes two central themes:

1) ensure robust product protection through adequate design and control of equipment and facilities; and

2) ensure that the operational and raw material inputs are predictable through adequate quality control and quality assurance. (GARY H. n.d.)

Physicochemical properties:

a) Protein solubility

According to the measurement conditions, two different types of protein solubility are distinguished .
1)True or thermodynamic solubility : refers to the concentration of the protein in solution, which is in equilibrium with a crystalline solid phase of that protein .
2) Apparent solubility: corresponds to the concentration of protein in a solution, which is in equilibrium with a solid amorphous precipitate of the same protein.
True solubility is a thermodynamic characteristic of a protein involving the chemical potential of the molecule. When the chemical potential of the protein molecule in solution becomes greater than its chemical potential in solid phase, protein precipitates. Given that solutions exhibit non ideal behaviour in the presence large molecular solutes, the chemical potential of the protein in solution $\mu2(\text{solution})$is related to the protein activity.

$$\mu2(\text{solution}) = \mu2°(\text{solution}) + RT \ln (\gamma2\ c2)$$

where R is the gas constant, T is the absolute temperature, $\mu2°(\text{solution})$ is the standard chemical potential, $\gamma2$ is the activity coefficient and c2 is the protein concentration in g·ml-1. Considering that protein in a crystalline phase is in thermodynamic equilibrium with the same protein in solution, equation 1 gives:

$$\mu2(\text{crystal}) = \mu2°(\text{solution}) + RT \ln (\gamma2\ S)$$

where $\mu2(\text{crystal})$ is the chemical potential of the protein crystal and c2 has been replaced by the protein solubility S (g·ml-1). Defining $\Delta\mu2 = \mu2°(\text{solution}) - \mu2(\text{crystal})$, protein solubility can be expressed as follows:

$$\gamma2\ S = \exp (-\Delta\mu2 / RT)$$

Since $\Delta\mu2$ cannot be experimentally determined, absolute protein solubility cannot be predicted. The hanging drop technique is one of the more common approaches to determine the solubility limits of crystalline protein.

b) Protein association and aggregation

Protein self-association denotes the formation of Small , soluble oligomers from a native species that are reversible upon simple dilution with buffer, , whereas protein aggregation depicts any formation of irreversible oligomers from non-native species.

Self-association
Self-association corresponds to the formation of dimers or oligomers from a native species resulting from relative weak non covalent protein interactions as for example observed in a monoclonal antibody (MAb). The reversibility can be indicative for the presence of an equilibrium between monomer and oligomer species that is shifted by changing the formulation conditions such as pH or decrease in protein concentration. Although their formation is reversible, associated species may have further consequences. Indeed, the associate re-dissolution depends on their rate of dissociation, which can be too slow for clinical use. In addition, reversible associates can lead to the formation of irreversible aggregates during storage by formation of covalent.

Aggregation
In contrast to self-association, aggregation is irreversible. Two main pathways conduct to protein aggregation. On the one hand, physical aggregation results from the noncovalent association of protein molecules that do not present changes in primary structure. On the other hand, chemical association occurs by formation of new covalent bonds between protein molecules. The most common chemical pathway lies on the formation or the exchange of disulfide bonds. Physical protein aggregation results from the association of unfolded proteins. The native protein is transformed in a transitional protein species that is prone to associate and to form protein aggregate occurs in three step:
1) Protein unfolding,
2) Association of unfolded monomers in oligomers,
3)Nucleation, growth and condensation in aggregates.

Factors inducing protein aggregation / association:

External factors: It includes shaking, stirring, pumping, freezing / thawing or heating.

Internal factors: It includes protein concentration in solution. Protein aggregation is concentration dependant, as increasing the protein concentration increases the total volume occupancy by protein molecules.

c) Hydrogen bonding

In proteins hydrogen bonds are formed between the backbone oxygen's and amide hydrogen. Hydrogen bonds are strong forces. They produce interatomic distances shorter.

Hydrogen bond have an important impact on protein structure, by stabilizing the protein secondary structure. However, interfacial hydrogen bonds are weaker than the intra-chain ones because of the hydrophilic surface of proteins and the rigid protein body binding. More water molecules mediate hydrogen bonds at the interfaces and thus control protein-protein interaction. (Gerhard W. 2009)

d) Stability problem

Protein stability is another common problem in protein expression. . It is also an important topic in purification, formulation, and storage. Properly folded proteins are usually stable during expression and purification. Sufficient amount of intact protein should be obtained. However some proteins appear to be unstable during expression and purification. Some of them are so unstable that sufficient amount of protein cannot be obtained. . Many factors such as amino acid sequence of the protein, protein construction, host cell strain, expression and purification conditions may affect protein stability.

a) Physical instability

1) Denaturation
2) Adsorption
3) Aggregation
4) Precipitation

b) Chemical instability

1) Hydrolysis
2) Oxidation
3) β-elimination
4) Racemization
5) Isomerization
6) Disulphide exchange

a) Physical instability:

1) Denaturation: Denaturation is unique to protein and occurs when their native tertiary and secondary structures are disrupted.

Preventive measures: It can be prevented by the use of the additives such as salts.

2) Adsorption

Protein exhibit a certain degree of surface activity due to that protein adsorb to surface of containers. Consequently biological activity may be lost if such absorption occurs during manufacturing and storage.

Preventive measures:

- Modify the surface of glass container.
- Add surfactant.
- Add macromolecules.

3) Aggregation

Certain protein tends to undergo self association resulting in the formation of multimers. Protein aggregation is the mainly caused by hydrophobic interaction resulting from denaturation. Preventive measures: it can be prevented by the use of surfactant, polyols or sugars.

b) Chemical instability:

1) Hydrolysis

Hydrolysis or deamidation occurs with protein contain susceptible Asn and Gln amino acid these are undergo deamidation to form free carboxylic acid. Neutral and alkaline conditions enhanced the rate of deamidation.

Preventive measures: Hydrolysis can be reduced by reducing the pH.

2) Oxidation

Proteins containing methionine, cystein, histidine may be sensitive to oxidation. Methionine and cysteine may lead to disulfide bond formation and loss of biological activity.

Preventive measures:

- Antioxidant: e.g. sodium sulfide, chelating agent,thiols etc.
- Elimination of peroxides.
- Prevention by using thiol scarvengers.

3) β –elimination:

At temperature above ambient temperature, proteins undergo disulfide cleavage due to B-elimination from cysteine residues. Such reactions occurs at low temperature and high pH.

Preventive measures:

By using thiol scarvengers such as copper ion, diethylmaleimide.

5) Racemization

Naturally occurring amino acids are in the L form isomerise into D form leads to the change in structure and therefore the activity of the proteins.

Barriers to protein drug delivery:

The oral route of administration for these substances is even more problematic because, in addition to proteolysis in the stomach, the high acidity of the stomach destroys them before they reach the intestine for absorption. Polypeptides and protein fragments, produced by the action of gastric and pancreatic enzymes, are further cleaved by exo- and endopeptidases in the intestinal brush border membrane to yield di- and tripeptides, and even if proteolysis by pancreatic enzymes is avoided, polypeptides are subject to degradation by brush border peptidases. Any of the peptides that survive passage through the stomach are further subjected to metabolism in the intestinal mucosa, where a penetration barrier prevents entry into cells. On the other hand, oral and colonic delivery have the advantage of delivering drugs through the intestinal tract to the hepatic portal vein, and then to the systemic circulation

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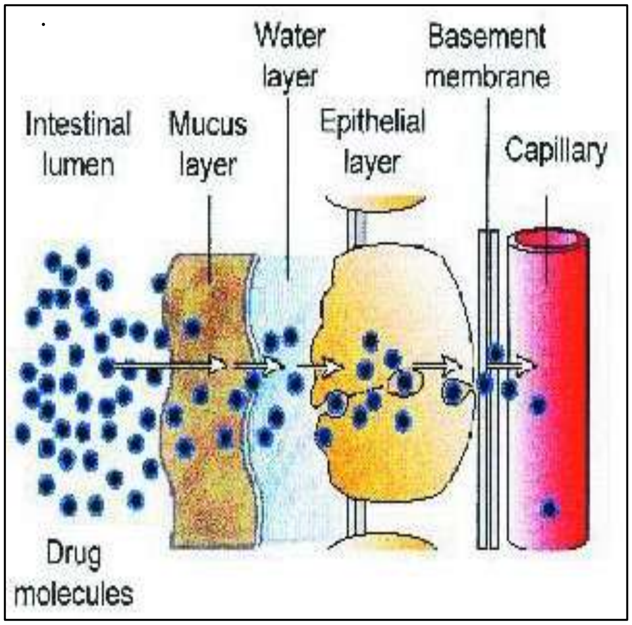

Fig: 2. Barriers to absorption of drug in intestine.

The ease of administration and higher degree of patient compliance with oral dosage forms are the major reasons for preferring to deliver proteins and peptides by mouth.

Pharmaceutical proteins are absorbed through the intestinal mucosa, although in minute amounts . Small amounts of peptide drugs can be absorbed by the action of specific peptide transporters in the intestinal mucosa cells; this suggests that properly formulated protein or peptide drugs may be administered by the oral route with retention of sufficient biological activity for their therapeutic use.

Strategies for oral delivery

Formulating for delivery through the gastrointestinal GI) tract requires a multitude of strategies. The dosage form must initially stabilise the drug, while making it easy to take orally. Once delivered to the stomach or intestinal tract, the protein has to be protected from enzymatic degradation since digestive processes are designed to break down proteins and peptides without any discrimination in favour of therapeutically active compounds. One strategy for overcoming the body's natural processes is to alter the environment for maximum solubility and enzyme stability of the protein by using formulation excipients such as buffers, surfactants and protease inhibitors. If the enzyme attack can be defeated or delayed, the proteins can be presented for absorption. Earlier work by Abuchowski and Davis (6) showed that proteins and peptides could be derivatised with polyethylene glycol (PEG) to achieve properties such as retention of activity, prevention of immunogenicity and prevention of excessive enzymatic degradation. More recently, Nobex has introduced the use of oligomers with both hydrophilic and lipophilic properties (7); these oligomers confer the enzymatic stability necessary for proteins to survive the digestive processes in the gut. Another strategy for oral delivery, therefore, is to promote absorption through the intestinal epithelium. Absorption may be enhanced when the product is formulated with acceptable safe excipients (8). A typical transport mechanism for proteins across the epithelial boundary is paracellular transport. There are tight junctions between each of the cells in the epithelium that prevent water and aqueous soluble compounds from moving past these cells. A number of absorption enhancers are available that will cause these tight junctions to open transiently, allowing water-soluble proteins to pass. Fatty acids, surface-active agents, EDTA, glycerides and bile salts have all been shown to be effective in opening these tight junctions. (Dr Soltero R. n.d.)

Route of administration:

1) Parenteral systemic delivery
a. Intravenous route
b. Intramuscular route
c. Subcutaneous route
2) Non- Parenteral systemic delivery
  a. Oral route
  b. Nasal route
  c. Buccal route
  d. Ocular route
  e. Rectal route
  f. Transdermal route
  g. Pulmonary route

1) Parenteral systemic delivery

Protein and peptides are mostly delivered by parenteral route as it provides the best choice for efficient delivery of the drugs.

Devices to deliver parenteral dosage forms:

Metered dose pens measured dose syringes

Fig: 3. Metered dose pen syringe.

a) Intravascular route:

The drugs are administered either by intravenous or intra-arterial route but most of the times IV route is preferred as administration through intra-arterial route is difficult.

Advantages: Drugs that are highly metabolized and are highly bound to tissues by IM route are basically given by this route.

EX: INSULIN, INTERFERONS AND ANTIBIOTICS.

Disadvantages: Causes pain, tissue necrosis and thrombopenia.

b) Intramuscular route:

The drug is injected in the muscle. Various drugs that are used for making depots are also administered by this route.

Advantages: Drugs that are administered for making depots and Better bioavailability for protein bound drugs.

Disadvantages: This route is not used for drugs that are metabolized at the site of administration.

Ex. of the drugs given by this route are LHRH, GH & LONG ACTING INSULIN

c) Subcutaneous route:

Drugs are administered under the fold of skin. In case of proteins and peptides, implanted polymeric devices are used. These devices are made by cross-linking the polymers and these polymers must be biocompatible and biodegradable.

Examples: Poly(d,l- lactide co-glycoside), Poly lactic acid Some of the drugs used are ACTH, CALCITONIN, INSULIN, BOVINE

2) Non- Parenteral systemic delivery

a) Oral route:

The oral route is by far the most popular route of drug administration but still suffers with drawbacks as far as protein and peptide delivery is concerned.

These include-

I) Acid catalysed degradation in stomach
II) Poor permeability across the gastrointestinal 1 mucosa
III) Proteolytic breakdown in the gastrointestinal tract
IV) First pass metabolism during transfer across absorption barriers in the liver.

The effectiveness of the pathway is severely compromise by

a) The limited efficiency and capacity of this absorption pathway.

b) The times of onset of pharmacological response due to kinetics of processing within lymphoid tissue and slow rate of lymph .

c) The potential loss of drug to local lymphocytes and microphages or through transport to mesenteric lymph nodes for processing there. oral administration is the most convenient ,provided that the drug is protected from the proteolytic degradation in the GI track and is transported intact from the intestinal lumen to the blood compartment. (Jain N.K. 2005)

b) Nasal route

Nasal cavity as potential route for drug delivery .The existence of an extensive microcirculation network underneath the nasal mucosa allows the effective systemic absorption of protein drugs ,which are susceptible to GI degradation when administered orally. Furthermore the fact that the drug molecules absorbed nasally can directly enter the systemic circulation before the hepatic circulation should be of potential benefits especially in case of drugs that are subject to extensive “first-pass” metabolism.

For those protein and peptides being investigated for delivery via inhalation, the ultimate site of action may be the airway surface(DNase) the airway cells (e.g. cyclosporin) or the systemic circulation (e.g. insulin).only protein for inhalation currently available in the market is DNase. (chow et al 2001)

Advantages of Nasal Drug Delivery System:

1. Drug degradation is absent.
2. Hepatic first – pass metabolism is absent.
3. Rapid drug absorption.
4. Quick onset of action.
5. The bioavailability of larger drug molecules can be improved. by means of absorption enhancer or other approach.
6. Better nasal bioavailability for smaller drug molecules.
7. Drugs which cannot be absorbed orally may be delivered to the systemic circulation through nasal drug delivery system.
8. Convenient route when compared with parenteral route for long term therapy. (chow et al 2001)

Limitation

1. The absorption enhancers used to improve nasal drug delivery system may have histological toxicity which is not yet clearly established.
2. Absorption surface area is less when compared to GIT.
3. Once the drug administered cannot be removed.
4. Nasal irritation (chow et al 2001)

c) Buccal routes

Modest success has been achieved by administering peptides from the buccal cavity,because of rich buccal vascularity, drugs delivered by the buccal route gain direct access to the systemic circulation and are not subject to first-pass metabolism. Also, therapeutic agents do not come in contact with the acidic digestive fluids secreted by the gastrointestinal tract . The buccal mucosa contains intercellular lipids which are responsible for its physical barrier properties, resulting in poor permeability for larger drugs, especially for peptides and proteins. Transfer of peptides with molecular weights above 500 - 1000 Da through buccal mucosa would require use of an absorption enhancer, however, an absorption enhancer, such as salicylate or a surfactant,is needed to deliver proteins/peptides across the mucosal tissues.

Various strategies employ for buccal delivery…

1) Adhesive tablet: e.g. adhesive tablet based on hydroxypropylcellulose.
2) Adhesive gels: by using polyacrylic acid and polymethacrylate.
3) Adhesive patches: e.g. protirel in HEC patches and buserellin.
4) Adhesive promoters: e.g. SLS, sodium myristate, bile acid, sodium glycocholate.

Advantages:

1) Relative to the nasal and rectal routes, the buccal mucosa has low enzymatic activity, and drug inactivation owing biochemical degradation is not as rapid and extensive.

2) Excellent accessibility to the buccal mucosa makes application of the dosage form painless, precisely located, and easily removable without discomfort at the end of the application period.

3) In addition, the buccal route may be useful for unconscious patients and in patients who have recently undergone surgery.

Disadvantages:

1) The surface area available for absorption in the buccal mucosa is much smaller than the gastrointestinal, nasal, rectal, and vaginal mucosae.

2) The buccal mucosa is continuously bathed by saliva, and the secreted saliva lowers the drug concentration at the absorbing membrane.

d) Ocular route

Ocular delivery of drugs is typically for the treatment of ocular inflammation, corneal wounds, and glaucoma. In addition, this route has been investigated for the systemic delivery of peptides and proteins. Already in 1931, ocular administration of insulin produced sustained lowering of the blood glucose level in proportion to the dose instilled. However, today it is known that the majority of the systemic drug absorption after ocular instillation takes place across the nasal mucosa after drainage via the nasolachrymal duct. Some absorption occurs also from the conjunctival sac. Drug absorption via the cornea is relatively low due to the lipophilicity of the corneal epithelium, dilution of the drug in the tear fluid (reflex tearing and reflex blinking) and drug binding to proteins in tear fluid. In addition, the corneal and conjunctival tissues act also as enzymatic barrier, which contain e.g. proteases. Therefore, the eye offers no additional advantage over the nose as systemic drug delivery site and is of higher interest only for drug administration for local (ophthalmic) therapy. The polypeptide antibiotics like cyclosporine,tyothericin, gramicidinen have often considered as potential candidates for achieving local pharmacological action in the eyes. (Jain N.K. 2005) (chow et al 2001)

e) Rectal route

The lower digestive tract is less harmful to administered drugs than the stomach and the small intestine due to the lower enzymatic activity and neutral pH. Also the rectal route of drug administration is safe and convenient.

The rectal delivery offers many advantages including…

a. Avoidance of a drug dilution prior to reaching the systemic circulation and of drug contact with digestive fluids and consequential degradation.

b. Reduction in firstpass metabolism.

c. Rapid systemic absorption.

d. Safe and convenient especially in case of neonates and infants. (Jain N.K. 2005) (chow et al 2001)

f) Transdermal route

Although the most common example of drug delivery using this route is nicotine and scopolamine patches, this route does not facilitate easy absorption of many drugs. Approaches for peptidergic drugs are iontophoresis, phonophoresis, application of penetration enhancers,prodrug and electroporation. Iontophoresis is the novel noninvasive method closely investigated for local and systemic delivery of peptides/proteins where an electric current is used to drive the molecule across the skin surface. Many studies have reported to encouraging result on the transdermal transport of insulin using iontophoresis technology. However the rate of transdermal iontophoretic delivery of peptide and protein drugs have been reported to be dependant on ph, ionic strength and electrolyte concentration of the formulation as well as on the applied voltage.

In phonohoresis, the absorption is enhance via thermal effect of ultrasonic waves and subsequent alteration in the physical structure of the skin. Penetration enhancers like dimethyl sulfoxide, oleic acid, surfactant,a zones and terpens etc.have been used to deliver a peptide/protein delivery through the skin. Prodrug with modeled characteristics permeated well across the skin. Recent advances in transfersoms, which are phosphatidyl choline based supermolecular aggregates designed to be sufficiently deformable to cross the intact skin barrier.

g) Pulmonary route

Deep lungs is an attractive site for protein delivery for two principle reasons

1) The relatively large surface area of the lungs which is approximately 100m^2 in the typical adult and

2) Rapid absorption of the drug into the blood stream through the alveoli. This concept of administration of the drug directly to the deep space has the advantage of direct absorption of the drug into the blood stream without degradation in the GI tract.dura and inhale have developed dry powder delivery systems by using mechanic dispersion and high speed compressed air ,respectively, to dispersed a protein powder into particles of the required size. These particles are small enough to reach the deep pulmonary space.

The lung offers a number of advantages which render it also a suitable organ for systemic drug delivery:
1) A large surface area of about 100 m^2 and an extremely well vascularized, thin epithelium. Thus, various drugs including peptides and proteins (e.g. insulin, human growth hormone, luteinizing hormone releasing hormone analogue, glucagon, calcitonin) have efficiently been delivered via the lung. Number of technologies for the delivery of drug formulations have been developed

(i) Pressurized metered dose inhalers using propellants to deliver micronized drug suspensions (Autohaler®, Spacehaler®),
(ii) Dry powder inhalers which dispense micronized drug particles with / without carrier (lactose) by inhalation activation (Spinhaler®, Rotohaler®, Diskhaler®), and
(iii) Nebulizers and aqueous mist inhalers which aerosolize drug solutions using compressed air or ultrasound (AERx®,Respimat®).
Although the pulmonary route of administration is very promising and the available delivery technologies are highly sophisticated, systemic drug delivery via the lung is still a challenging area of research. A key issue is the achievement of high efficiency to the alveolar region. (Jain N.K. 2005)

Analytical methods for protein characterization

A. Primary structure and sequence

The verification of amino acid sequence on the protein level is most often achieved by peptide mapping and characterization (sequencing) of the isolated peptide by mass spectroscopic (MS) analysis.
Verification of amino acid sequence by
1) Peptide mapping and
2) Mass spectroscopy

1) Peptide mapping

Peptide mapping may be used to determine Determination of Oxidation, De-Amidation, Acetylation, Proteolysis, Phosphorylation, Disulfide Bonding and Free Sulfhydrol groups, by using the following techniques.
Peptide mapping by -

- HPLC
- LC/MS

2) Mass spectroscopy [FAB-MS/LC/MS]

The high specificity of certain proteolytic enzymes in cleaving polypeptide chains only at certain residues results in a very characteristics of peptide.
Ex. Tripsine, cleaves proteins only at lysine and arginine residues resulting in peptides that end either with lysine or arginine (except the C terminal peptide), and the same pattern will be obtained for each digest of a given protein. These peptides are then resolved by reverse phase hi performance liquid chromatography (RP HPLC). The HPLC pattern, therefore, results in a” fingerprint” that is characteristic for a given protein. The individual peptides of a protein can be isolated from the HPLC profile and further characterized by mass spectroscopic analysis.

B. Secondary and tertiary structure

Changes in the conformation of proteins lead to aggregation, decrease in biological activity and in some cases immunogenicity. Therefore, the design of a formulation for optimum stability of proteins must consider all aspects of the structure of a biopharmaceutical. Methods for evaluating secondary structure are primarily physical, spectroscopic methods.
The secondary structure can be evaluated by a technique known as **circular dichroism (CD).** Aminoacides (except glycine) are asymmetric owing to the presence of the chiral carbon. The optical properties of polypeptides are due to the asymmetric centers of its constituent amino acids. Polypeptides, thus, interact differently with right and left circulatory polarized light. CD is a technique that measures the unequal absorption of left and right circulatory polarized light.

Fourier transform infrared spectroscopy (FT-IR) has also provided an estimate of secondary structure composition. FT-IR also has the advantage of being able to evaluate the aspects of protein structure in the solid state.
Many protein exhibit **fluorescence** in the 300-400 nm range when excited at 250-300 nm, as result of the presence of aromatic amino acids (Trp, Tyr, Phe).Ex. A buried tryptophan is usually in a hydrophobic environment and will fluorescence with a wavelength maximum in the 325-330 nm range, while an exposed

residue (or free amino acid) fluorescence at around 350-355 nm.

C. Protein glycosylation

This HPAC-mapping procedure , which can detect even small changes in protein glycosylstion pattern, separates different structures that coelute using HPAEC-PAD and is particularly suitable for routine testing of glycosylation consistency in glycoprotein pharmaceuticals.

D. Protein concentration

a) Colorimetric method: One of the most fundamental measurements made on a protein is the determination of concentration. Peptides and proteins that do not absorb visible light can react with reagents to form colored compounds. The widely used reagent,

1) Ninhydrin reacts with amino groups of amino acids and peptides to produce an intensely colored product that has a maximum absorbance at 570 nanometer.

2) In the biuret reaction of copper, resulting blue product is quantitated at 540-560nm .

b) Ultraviolet absorption spectroscopy : The absorption spectrum of a protein in the UV wavelength range is a net result if absorption of light by the carbonyl group of the peptide bond (190-210 nm),the aromatic amino acids (250-320 nm), and the disulfide bond (250-300 nm).

E. Surface charge

a) Gel electrophoresis:

Polyacrylamide gels may be have many different constant or gradient compositions. Proteins may be run directly, or are denatured, reduced, and alkylated. Samples, with a visible dye added, are loaded in wells cut into the top of the gel.

Loading capacity depends on gel size and thickness. In 2D IEF/PAGE, the gel strip from IEF is loaded into a single large well.

Molecular weight standards are often run to calibrate the gel. After separation, the gel is removed from the rig and stained, or bands are blotted onto a membrane. (Maginley M. 2008)

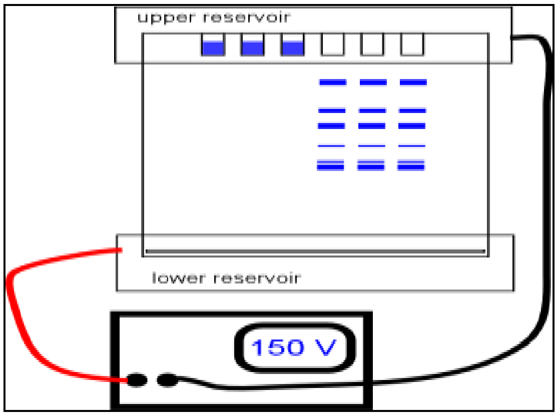

Fig: 4 Gelelecrophoresis

b) Isoelectric focusing

The charge of a native protein is dependent on the pH of the solution. At the isoelectric point (PI),the protein's net charge will be zero and the mobility in the electric field will be zero. Thus, if the electric field is also pH gradient, the protein will migrate to the point at which the pH is the same as the PI and the migration will stop. This phenomenon is known as isoelectric focusing.

In a pH gradient, under an electric field, a protein will move to the position in the gradient where its net charge is zero. An immobilized pH gradient is created in a polyacrylamide gel strip by incorporating a gradient of acidic and basic buffering groups then the gel is cast. Proteins are denatured, reduced, alkylated and loaded in a visible dye. The sample is soaked into the gelalong its entire length before the field is applied.

Resolution is determined by the slope of the pH gradient and the field strength.

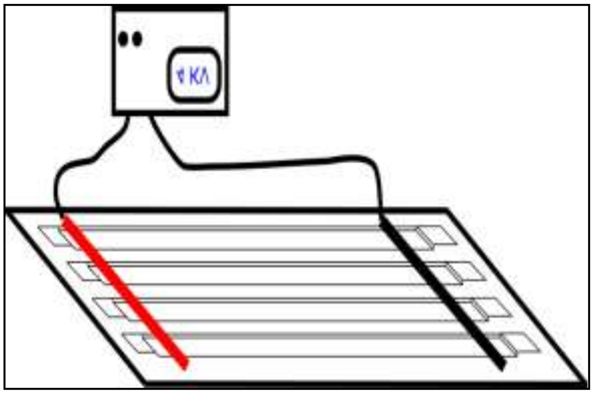

Fig: 5.Isoelecric focusing

c) Ion exchange chromatography

A protein with net positive charge tense to bind to a matrix with a net negative charge by ionic interaction and vice versa the charge distribution on the surface of a given protein is dependent on the solution pH, which indicates the binding and elution conditions of the ion exchange chromatography method. In this method, the sample is loaded onto an ion exchange column at a pH where it binds. The column is then eluted with an ampholyte or buffer mixture selected to generate a pH gradient that gradually flows down the column. When the PI of a bound protein is reached, that protein is released from the resine. (Barbara M. 1996)

F. Protein size

a) polyacrylamide gel electrophoresis with the denaturating agent sodium dodecyl sulfate (SDS-PAGE)

The SDS molecule carries a negative charge, and complexes of proteins with SDS shows free electrophoretic mobility. When a mixture of SDS–saturated proteins is electrophoresed in a gel matrix with a correct pore size, the major factor determining there migration rate in there effective size.

The apparent molecular weight of protein can also be determine by gel filtration or size exclusion chromatography, ESI-MS (electro spray ionization MS) and MALDI-MS (matrix assisted laser desorption ionization MS mass spectroscopy.

G. Bioassay, Potency assay:

In vivo: bioassay of h-GH

In vitro: murine thymocyte proliferation assay analysis of h-IL-1.

Potency assay: clot lysis assay of r-TPA.

H. Product purity and safety testing

1) Limulus amoebocyte lysate (LAL)
2) Virus test
3) DNA assay
4) ELISA
5) Ultra filtration (Garidel P. 2010)

Recent advances:

Recent advances in protein drug delivery system

1. Polymeric drug delivery system
2. Microsphere encapsulation
3. Polymer scaffolds
4. Magnetic targeted carriers
5. Liposome’s
6. Hydrogel based drug delivery

1. Polymeric drug delivery system

Advantages

- Frequency of dose reduced
- Drug utilized more effectively
- Drug stabilized inside the polymer matrix
- Reduced side effect

Disadvantages

- Possibility of dose dumping.
- Deactivation of drug inside polymer.
- Polymer should be biodegradable ,biocompatible. Example of such polymers are polylactide/glycosides, polyanhydride, and polyphosphate. Diffusion of drug out of polymer occurs according to ficks law of diffusion. Drug releases concentration dependant it is less applicable for larger molecules.

Polymer is degraded by hydrolysis or enzymatic (phoshatases , proteasases, etc)

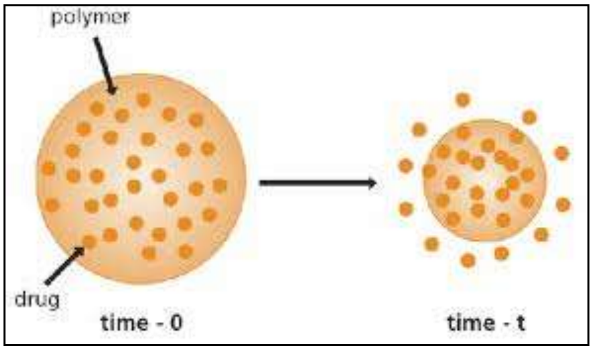

Fig: 6. Polymer degradation and drug release

2. Microsphere encapsulation (size-100μm)

Encapsulation is the process involves encapsulating protein and peptide drugs in small porous particles for protection from” insunts” and for sustaind release.

There are two types of microspheres

1) Biodegradable

2) Non-biodegradable

Biodegradable are prepared from biodegradable polymer like gelatin, polilactic-co-glycolicacid (PLGA).

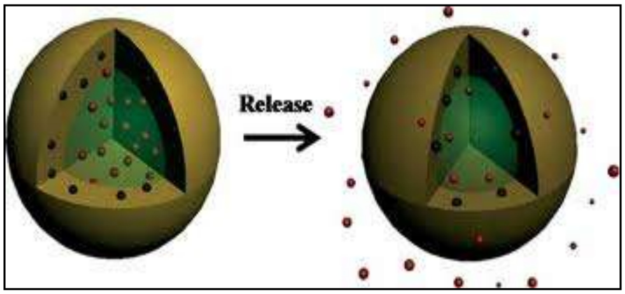

Fig: 7. Drug release from microspheres.

The **hydrophilic polymer microsphere** like gelatin is used for burst release of drug. **Hydrophobic polymer microsphere** like PLGA is used for sustained release of drug but it denaturates the protein. **Hybrid polymer microsphere** are used for good sustained release and it keeps protein active/native.

3) Polymer Scaffolds:

Incorporate drug into polymeric matrix. Protection of drug from enzymatic degradation – particularly Applicable to peptide and protein drugs Release drug at known rate over prolonged duration Drug dispersed or dissolved in suitable polymer Release - diffusion of drug through polymer - diffusion through pores in polymer structure – therefore different release profiles result(dissolved or dispersed) Polymer Scaffold.

4) Magnetic Targeted Carriers (MTCs):

Magnetic Targeted Carriers (MTCs) Microparticles, composed of elemental iron and activated carbon Drug is adsorbed into the MTCs and transported The drug attaches to the carbon component The particles serve as delivery vehicles to the area of the tumor for site-specific targeting.

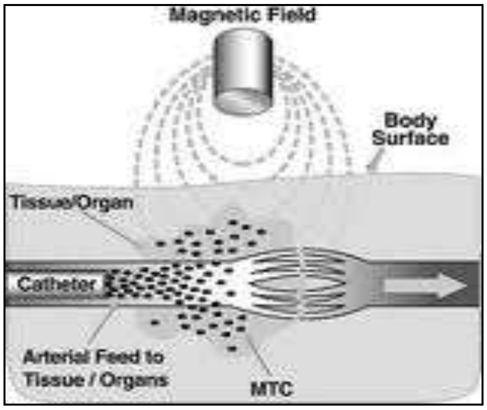

Fig: 8 Magnetic targeted carriers.

5) Liposomes:

Liposomes are Hydrophobic and Hydrophilic Spherical vesicles with a phospholipid bilayer. Potential of Liposomes in drug delivery has now realized. Bloemycin encapsulated in thermo sensitive Liposomes enhanced antitumor activity and reduced normal tissue toxicity. S.C injection of negatively charged Liposomes produced a prolonged hypoglycemic effect in diabetic dogs Liposomes have recently been used successfully as vehicles for vaccines.

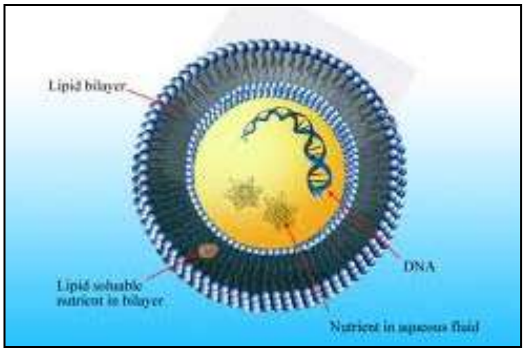

Fig:9. Liposome.

5) **Hydrogel Based Drug Delivery:**

Hydrogels are three dimensional networks of hydrophilic polymers that are insoluble. Hydrogels can swell as a result of changes in pH, Temp., ionic strength, solvent composition, pressure and the application of electric fields Hydrogel Based Drug Delivery Insulin has been one drug that has been incorporated in Hydrogels and investigated by researchers extensively.

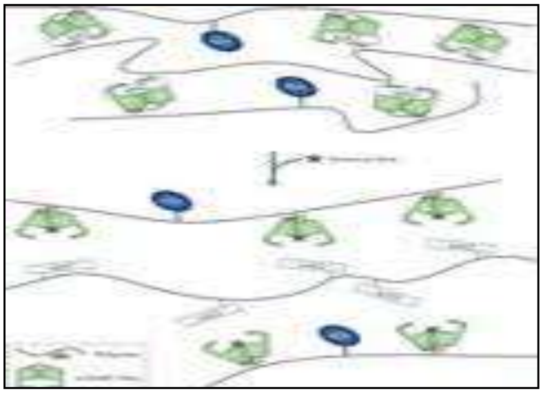

Fig: 10. Hydrogel based drug delivery system

Marketed formulations:

Calcitonin Salmon Nasal Spray

Calcitonin salmon is used to treat osteoporosis in women who are at least 5 years past menopause and cannot or do not want to take estrogen products. Osteoporosis is a disease that causes bones to weaken and break more easily. Calcitonin is a human hormone that is also found in salmon. It works by preventing bone breakdown and increasing bone density (thickness).

Calcitonin salmon comes as a spray to be used in the nose. It is usually used once a day, alternating nostrils every day.

Ex. Miacalcin®

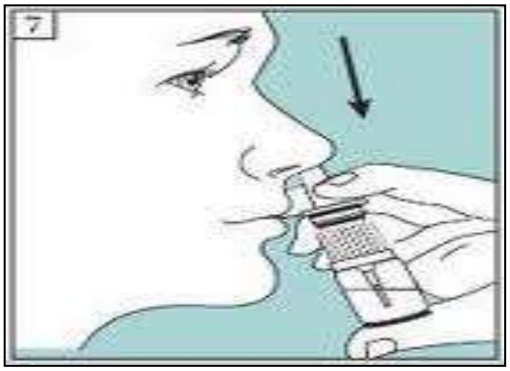

Fig: 11. Administration of calcitonin salmon by nasal spray.

Desmopressin Nasal spray

Ex. DDAVP®(Desmopressin Acetate) nasal spray

Nasal desmopressin is used to control the symptoms of a certain type of diabetes insipidus ('water diabetes'; condition in which the body produces an abnormally large amount of urine). Nasal desmopressin is also used to control excessive thirst and the passage of an abnormally large amount of urine that may occur after a head injury or after certain types of surgery. One brand of desmopressin nasal spray **(Stimate®)** is used to stop bleeding in people who have certain bleeding disorders.

Insulin

A natural hormone made by the pancreas that controls the level of the sugar glucose in the blood. Insulin permits cells to use glucose for energy. Cells cannot utilize glucose without insulin.

Types of Insulin: Currently, insulin is available from bovine (beef), porcine (pork), and recombinant (human) sources. Regular (rapid onset, short-acting) and NPH (slower onset, longer duration) human insulin are the most commonly-used preparations. An ultrarapid-acting insulin analog, insulin lispro injection (Humalog(r)) is a chemically-modified, natural insulin. It was approved by the FDA in June, 1996.

Insulin is administered by injection via the subcutaneous (under the skin) route. Regular insulin acts within 30 minutes, and its effects last 6 to 8 hours. Insulin can be administered in S.C tissue by pen or jet injector.

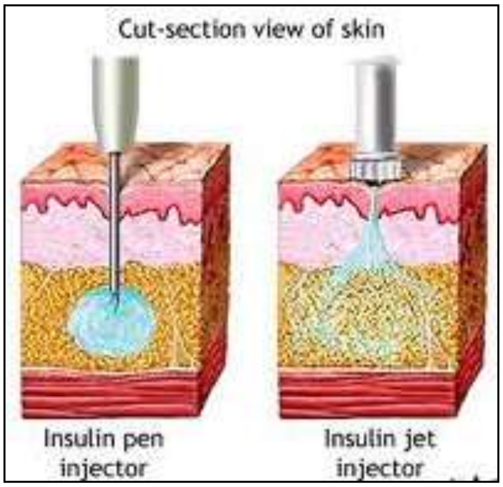

Fig: 12 Insulin administration by pen and jet injector

Application and Market:

1. In Disease:

During past few decades, therapeutic proteins have emerging in prominence as potential drug candidates for the future to treat patients suffering from various diseases like cancers, heart attacks, strokes, cystic fibrosis, diabetes, anemia, hemophilia, Gaucher’s disease etc. proteins play critical roles in cell biology and they have many potential therapeutic applications in preventing and curing various diseases like diabetes mellitus. (Dulal P. 2010)

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2. Local delivery:

For the natural treatment of topical nasal disorders the drug is administered through nasal route. Among the most common examples are antihistamines and corticosteroids for rhinosinusitis, and nasal decongestants for cold symptoms.

3. Systemic delivery

The intranasal administration of drugs is an effective way for systemic availability of drugs as compared to oral and intravascular routes. Actually, it seems to present fast and extended drug absorption, and it has been supported by many studies planned to compare intranasal drug delivery against oral and parenteral administration. Examples include analgesics (morphine), cardiovascular drugs as propranolol and carvedilol, hormones such as levonorgestrel, progesterone and insulin, anti-inflammatory agents as indomethacin and ketorolac, and antiviral drugs (acyclovir). Some examples which are available in the market include zolmitriptan and sumatriptan for the treatment of migraine and cluster headaches.

4. Nasal vaccines

Nasal mucosa is the first site of contact with inhaled antigens and therefore, its use for vaccination, especially against respiratory infections, has been extensively evaluated. In fact, nasal vaccination is a promising alternative to the classic parenteral route, because it is able to enhance the systemic levels of specific immunoglobulin G and nasal secretary immunoglobulin A. Examples of the human efficacy of intranasal vaccines include those against influenza A and B virus, proteosoma-influenza, adenovirus-vectored influenza, group B meningococcal native, attenuated respiratory syncytial virus and parainfluenza 3 virus.

5. CNS delivery through nasal route

Intranasal route has promising approaches for delivery of drugs to the brain. The delivery of drugs to the CNS from the nasal route may occur via olfactory neuroepithelium. The transport via trigeminal nerve system from the nasal cavity to CNS has also been described. Drug delivery through nasal route into CNS has been reported for Alzheimer's disease, brain tumors, epilepsy, pain and sleep. (Rahisuddin 2011)

Market

The success of protein and peptides therapeutics is revolutionizing the biotech and pharmaceutical market. Spurring the creation of the next generation product with reduced immunogenicity improve safety, and greater effectiveness. New technologies, genetic and chemical techniques will ensure a competitive edge in developing improved protein and peptide based therapeutic.

The report point out the protein engineering market in 2006 was worth $67 billion (10% of the total pharma sales) and is forecast to rise to $118 billion (12% of the total pharma sales) in 2011. Despite there remarkable success protein drug continue to suffer certain drawbacks especially with delivery (intravenously and subcutaneously injected). The past three years seen approval of product for non-parenteral delivery, alongside advances in protein and peptides drug delivery. The increase use, development and discovery of protein therapeutics will lead to increase opportunities for market. The protein therapeutic market is largely immediate release, but there is a trend moving in a sustain release formulations.

While majority of the protein therapeutics on the market do not have the devices available, this is the growing segment of the market. (Cindy H. 2009)

Peptide and protein drugs are rapidly becoming a very important class of therapeutic agents. Currently, they are mostly delivered by parenteral administration. Because they are extremely short-acting repeated injections are often required. Of non-parenteral routes, intranasal route is perhaps the most viable and favorable for chronic systemic medication of peptide drugs. As we look to what is on the horizon for new protein therapeutic proteins with important therapeutic application will emerge. We expect that with continued support, the medical and pharmaceutical field will be an important beneficiary of therapeutic proteins for years to come.

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INTRAOCCULAR DRUG DELIVERY SYSTEM

Maravaniya Pathikkumar, Zarikar Nitin, Pawar Yogesh

INTRODUCTION:

Ocular drug delivery systems are developed to treat eye locally, whereas past formulations are targeted to reach systemic circulation and these are designed to overcome all the disadvantages of conventional dosage forms such as ophthalmic solutions (al, 2011). The main problem with conventional dosage forms is eye irritation (due to drug particle size and shape) which induces lacrimation i.e. overflow on to lids, tear turn over, and due to pharmacokinetic responses like metabolism, non-specific binding and different mechanisms like diffusion, dissolution and erosion the conventional dosage forms are less advantageous (thakur, 2011).

The eye drop dosage form is easy to instill but suffers from the inherent draw back that the majority of the medication it contains is immediately diluted in the tear film as soon as the eye drop solution is instilled into the cul-de-sac and is rapidly drained away from the precorneal cavity by constant tear flow, a process that proceeds more intensively in inflamed than in the normal eyes, and lacrimal-nasal drainage. Therefore, only a very small fraction of the instilled dose is absorbed into the target tissues i.e. about 1.2% is available to the aqueous humor and relatively concentrated solution is required for instillation to achieve an adequate level of therapeutic effect (Menqi, 2004).

The frequent periodic instillation of eye drops becomes necessary to maintain a continuous sustained level of medication. If there is high drug concentration in eye drop solution it may give, the eye massive and unpredictable dose of medication as well as it creates greater loss of lacrimal-nasal drainage system. Subsequently this may lead to systemic side effects.

Most drugs for ophthalmic use like pilocarpine, epinephrine, local anaesthetics, atropine, etc are weak bases which are generally formulated at acidic pH to enhance stability. But due to their highly ionized form, ocular diffusion is poor. This, coupled with tear drainage, further reduces the rate and extent of absorption. Moreover, if the drug has short half-life, the problems become more complicated. Frequent dosing of large doses of such drugs becomes necessary to achieve the therapeutic objective which often results in corresponding increase in local and systemic side effects (Brahmankar, 2010). So research on Novel ophthalmic drug delivery systems is in progress to overcome all these disadvantages of conventional ophthalmic dosage forms.

ANATOMY OF EYE:

The eye consists of transparent cornea, lens, and vitreous body without blood vessels. The oxygen and nutrients are transported to this non-vascular tissue by aqueous humor which is having high oxygen and same osmotic pressure as blood. The aqueous humor in human is having volume of 300 μl that fills the anterior chamber of the eye which is in front of lens. It is shown in **figure 1.**

The cornea is covered by a thin epithelial layer continuous with the conjunctiva at the cornea-sclerotic junction. The main bulk of cornea is formed of criss-crossing layers of collagen and is bounded by elastic lamina on both front and back. Its posterior surface is covered by a layer of endothelium. The cornea is richly supplied with free nerve endings. The transparent cornea is continued posteriorly into the opaque white sclera which consists of tough fibrous tissue. Both cornea and sclera withstand the intra ocular tension constantly maintained in the eye (Henderer, 2006).

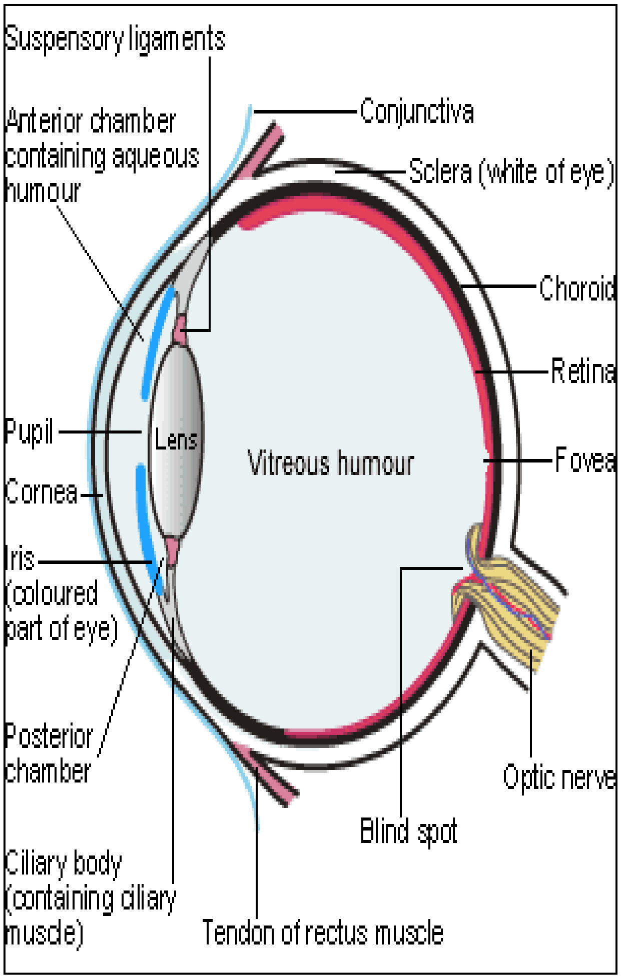

Fig.1. Anatomy of eye

The eye is constantly cleansed and lubricated by the lacrimal apparartus which consists of four structures.

lacrimal glands,

lacrimal canals,

lacrimal sac,

nasolacrimal duct

The lacrimal fluid secreted by lacrimal glands is emptied on the surface of the conjunctiva of the upper eye lid at a turnover rate of 16% per min. It washes over the eye ball and is swept up by the blinking action of eye lids. Muscles associated with the blinking reflux compress the lacrimal sac, when these muscles relax; the sac expands, pulling the lacrimal fluid from the edges of the eye lids along the lacrimal canals, into the lacrimal sacs. The lacrimal fluid volume in humans is 7 µl and is an isotonic aqueous solution of bicarbonate and sodium chloride of pH 7.4. It serves to dilute irritants or to wash the foreign bodies out of the conjuctival sac. It contains lysozyme, whose bactericidal activity reduces the bacterial count in the conjuctival sac (Chien, 2011).

The physiological barriers to diffusion and productive absorption of topically applied drug exist in the precorneal and corneal spaces.

The precorneal constraints that are responsible for poor bioavailability of conventional ophthalmic dosage forms are solution drainage, lacrimation, tear dilution, tear turn over and conjuctival absorption (Lee, 19993).

DISEASES OF EYE:

The eye is a sensory and sensitive organ which is located on the surface of the body, is easily injured and infected

According to the location of diseases, ocular disorders are grouped as

1. Periocular diseases,

2. Intraocular diseases.

The periocular diseases are explained as follows:

Conjuctivitis: It is a condition where redness of the eye and the presence of a foreign body sensation are evident. There are many causes of conjunctivitis, but the great majority are the result of acute infection or allergy. Bacterial conjunctivitis is the most common ocular infection.

Keratitis: The condition in which patients have a decreased vision, ocular pain, red eye, and often a cloudy/opaque cornea. Keratitis is mainly caused by bacteria, viruses, fungi, protozoa and parasites.

Trachoma: The conjunctival inflammation is called "active trachoma" and usually is seen in children, especially pre-school children. It is characterized by white lumps in the undersurface of the upper eyelid and by non-specific inflammation and thickening often associated with papillae. This is caused by the organism Chlamydia trachomatis. Active trachoma will often be irritating and have a watery discharge.

Dry Eye: If the composition of tears is changed, or an inadequate volume of tears is produced, the symptom of dry eye will result. Dry eye conditions are not just a cause for ocular discomfort where it also results in corneal damage.

Periocular diseases such as these are relatively easily treated using topical formulations.

The intraocular diseases are explained as follows:

One of the intraocular diseases is intra ocular infection which includes intraocular infections: i.e. infections in the inner eye, including the aqueous humor, iris, vitreous humor and retina. They are more difficult to manage and occur commonly after ocular surgery, trauma or may be due to endogenous causes. Such infections carry a high risk for damage to the eye and also afford the possibility of spread of infection from the eye into the brain.

Other common intraocular disease is glaucoma, considered to be one of the major ophthalmic clinical problems in the world. More than 2% of the population over the age of 40 have this disease, in which an increased intraocular pressure (IOP) greater than 22 mm Hg ultimately compromises blood flow to the retina and thus causes death of the peripheral optic nerves. This process results in visual field loss and ultimately blindness (Masood, 2007).
Apart from these common problems of eye are cataract and macular degeneration (Wilson, 2005) and sometimes diseases which may be of a systemic origin such as diabetes or hypertension effect the eye.

OCULAR DRUG DELIVERY SYSTEMS:

The necessary characters of Ideal control release ocular drug delivery system are:
It should not induce a foreign-body sensation or long lasting blurring.
It should possess more local activity than systemic-effects.
It must deliver the drug to the right place, i.e. in targeting the ciliary body.
It should be easy to self-administer.
The number of administrations per day should be reduced.
The primary approaches in the design of control release ocular drug delivery system attempt to slow down the drainage of drug by tear flow. The various formulations are explained as below.

A. Ophthalmic Solutions of Drug Resinates:

The first successful control release ophthalmic product for topical application using ion exchange resin technology for treatment of glaucoma was betaxolol ionic suspension (Betoptic S, 0.25%).

In this the drug is bound to Amberlite resin, a cationic exchange resin of sulphonic acid styrene-vinyl copolymer. The solution is also having carbomer(Carbopol 934P) which acts as an viscosity enhancer which helps in increasing the residence time of product in eye (Brahmankar, 2010).

B. Viscous solutions and Hydrogels

The principle involved in the formulation of viscous solutions and hydrogels is addition of hydrocolloids to aqueous drug solutions. Commonly used polymers in these formulations are cellulose derivatives, carbomers, polysaccharides, polyvinyl alcohol, polyvinyl pyrrolidone and recently, hyaluronic acid.

Gels permit longer residence time in the precorneal area than viscous solutions. Hence, the drug solution that gels in the conjuctival cul-de-sac and it is more acceptable. Hence these formulations are referred to as in-situ gel forming systems (S.P., 2011).

The mechanisms which form sol to gel transition in the conjunctival pouch are due to change in pH, temperature, or due to change in ionic environment.

E.g. Timolol formulation based on the gellan gum undergoes sol to gel transition due to the ionic content of the tears.

C. Mucoadhesive Formulations:

Mucoadhesion is based on entanglement of non-covalent bonds between polymers and mucous. Commonly used polymers in these formulations are many high molecular weight polymers with different functional groups like carboxyl, hydroxyl, amino and sulphate which are capable of forming hydrogen bonds and not crossing biological membranes. These have been screened as possible mucoadhesive excipients in ocular delivery systems [(Felt, 1999), (Menqi, 2004), & (Brahmankar, 2010)].

The Charged polymers i.e. both anionic and cationic polymers show better mucoadhesive capacity than the non-ionic ones

Anionic polymers: Sodium alginate, Poloxamer, Carbomer, Sodium Carboxy Methoxy Cellulose.

Cationic polymers: Chitosan.

This formulation helps in increasing the residence time of drug in the eye.

D. Dispersed Systems:

These are based on liposomes, nanoparticles or nanocapsules and the development of nanoproducts was very challenging (Ravi, 2008).

1. Lipsomes:

The potential advantages achieved with the liposomes are the control of the rate of encapsulated drug and protection of the drug from the metabolic enzymes present at the tear corneal epithelium interface.

Liposomes are vesicles composed of lipid membrane enclosing an aqueous volume. These structures are formed simultaneously when a matrix of phospholipids are agitated in an aqueous medium to disperse the two phases. Phospholipids commonly used are phosphotidylcholine, phosphotidylserine, phosphatidic acid sphingomyelins, and cardiolipins. They may be multilamellar vesicles or unilamellar depending upon the number of concentric alternating layers of phospholipid and aqueous phases (thakur, 2011).

They can be prepared by sonication of dispersion of phospholipids, reverse phase evaporation, solvent injection, detergent removal or calcium induced fusion. Lipophilic drugs are delivered to a greater extent to the ocular system by these liposomes.

The drawbacks associated with the liposomes in ocular drug delivery are due to short shelf life, limited loading capacity and obstacles such as sterilization of the preparation.

2. Nanoparticles:

This approach is considered mainly for the water soluble drugs. Nanoparticles are particulate drug delivery systems 10-1000 nm in the size in which the drug may be dispersed, encapsulated or absorbed.

Nanoparticles for ophthalmic drug delivery were mainly produced by emulsion polymerization. In this process a poorly soluble monomer is dissolved the continous phase which may be aqueous or organic [(Menqi, 2004), (Vyas, 2011)]. Polymerization is started by chemical initiation or by irradiation with gamma rays, ultraviolet or visible light. The emulsifier stabilizes the resulting polymer solution. The materials mainly used for the preparation of ophthalmic nanoparticles are polyalkylcyanoacrylates. The pH of the polymerization medium has to be kept below 3. After polymerization pH may be adjusted to the desired value. The drugs may be added, before, during or after the polymerization. The polymers used for the preparation of ophthalmic nanoparticles are rapidly bio-degradable. Hence the nanoparticles are very promising as targeted drug carriers to inflamed region of the eye.

E. Ophthalmic-Inserts:

Ophthalmic inserts are aimed at remaining for a long period of time in front of the eye (David, 2008). These solid devices are intended to be placed in the conjunctival sac and to deliver the drug at a comparatively slow rate.

The advantages of these systems are:

-Ocular contact time is increased.
-Accurate dosing is possible.
-Constant and predictable rate of drug release can be achieved.
-Systemic absorption can be reduced and side effects can be reduced.
-Increased shelf life can be achieved Better patient compliance.
-Targeting to internal ocular tissues can be done.

Some of the ophthalmic inserts are explained as follows:

1. Non-erodible ocular insert:

The Non-erodible ocular inserts include Ocusert, and Contact lens.

Ocusert was one of the earlier ocular inserts in use. The technology used in this is an insoluble delicate sandwich technology (S.P., 2011). In ocusert the drug reservoir is a thin disc of pilocarpine-alginate complex sandwiched between two transparent discs of micro porous membrane fabricated from ethylene-vinyl acetate copolymer (Patrick J., 2006). The micro porous membranes permit the tear fluid to penetrate into the drug reservoir compartment to dissolve drug from the complex.

E.g. Alza-ocusert: In this Pilocarpine molecules are then released at a constant rate of 20 or 40 µg/h for 4 to 7 days. Used in the management of glaucoma.

The use of pre-soaked hydrophilic contact lenses was used for ophthalmic drug delivery. Therapeutic soft lenses are used to aid corneal wound healing in patients with infection, corneal ulcers, which is characterized by marked thinning of the cornea.

An alternative approach to pre-soaked soft contact lenses in drug solutions is to incorporate the drug either as a solution or suspension of solid particles in the monomer mix (Kishan, 2011). The polymerization is then carried out to fabricate the contact lenses. This technique is promising longer release up to 180 h as compared to pre-soaked contact lenses.

Disadvantages of these non-erodible ocular inserts are

Complexity and difficulty of usage is noticed particularly in self administration.

Tolerability in the eye is poor, due to rigidity, size or shape (Horward, 2005).

Foreign body sensation and they are to be removed at the end of the dosing period.

2. Erodible ophthalmic insert:

The marketed devices of erodible drug inserts are Laciserts, SODI, and Minidisc.

a. **Lacisert:** It is a sterile rod shaped device made up of hydroxyl propyl cellulose without any preservative is used for the treatment of dry eye syndromes (rajasekaran, 2010). It weighs 5 mg and measures 12.7 mm in diameter with a length of 3.5 mm.

Lacisert is useful in the treatment of keratitis whose symptoms are difficult to treat with artificial tear alone. It is inserted into the inferior fornix where it imbibes water from the conjunctiva and cornea, forms a hydrophilic film which stabilizes the tear film and hydrates and lubricates the cornea (Menqi, 2004). It dissolves in 24 hours.

b. **Sodi:** Soluble Ocular Drug Insert is a small oval wafer developed for cosmonauts who could not use eye drops in weightless conditions. It is sterile thin film of oval shape made from acrylamide, N-vinylpyrrolidone and ethylacrylate called as

ABE (Menqi, 2004). It weighs about 15-16 mg.

It is used in the treatment of glaucoma and trachoma. It is inserted into the inferior cul-de-sac and get wets and softens in 10-15 seconds (Agrawal, 2011). After 10-15 min the film turns into a viscous polymer mass, after 30-60 minutes it turns into polymer solutions and delivers the drug for about 24hours.

c. **Minidisc:** The minidisc consists of a contoured disc with a convex front and concave back surface in the contact with the eyeball. It is like a miniature contact lens with a diameter of 4-5mm.

The minidisc is made up of silicone based prepolymer-α-ψ-bis (4-methacryloxy) butyl polydimethyl siloxane. Minidisc can be hydrophilic or hydrophobic to permit extend release of both water soluble and insoluble drugs

3. New Ophthalmic Drug Delivery System:

The New Ophthalmic Drug Delivery System (NODDS) is a method of presenting drugs to the eye within a water soluble drug loaded film. It provides accurate, reproducible dosing in an easily administered preservative free form (Brahmankar, 2010).

These systems were developed with two primary objectives:

a. To provide a sterile, preservative-free, water-soluble, drug loaded film to the eye

b. It serves as a unit-dose formulation for the delivery of a precise amount of drug to the ocular surface.

The basic design of NODDS consists of three components-

Water soluble, drug-loaded film (flag) attached via Thin, water soluble membrane film, to Thicker, water-soluble, handle film.

All the three films are made using the same grade of polyvinyl alcohol (PVA) in aqueous medium, but at three different concentrations.

The NODS is approximately 50 mm in length, 6 mm in width, the flag (drug loaded film) is semi circular in shape and has an area of 22 sqmm and a thickness of 20 µm and a total weight of 500 µg of which 40% can be drug.

On contact with the tear film in the lower conjunctival sac, the membrane quickly dissolves releasing the flag into the tear film (Singh, 2011). The flag hydrates allowing diffusion and absorption of the drug. For easy handling the handle film is sandwiched between the paper strips before the whole unit is sealed in a moisture free pouch. By this system an eight fold greater bioavailability was observed compared to the conventional eye drop.

4. Bio adhesive Ophthalmic Drug Inserts:

These are soluble inserts made of synthetic and semi synthetic polymers. They are composed of ternary mixture of hydroxypropylcellulose, ethylcellulose and carbomer (Carbapol 934P) [(Robinson, 1993), (Brahmankar, 2010)].

These are developed to overcome the drawback of available inserts which are sometimes displaced or expelled by eyeball movements.

These are rod shaped inserted obtained by the extrusion of a dried homogeneous powder mixture composed of the polymeric vehicle and the active compound using a specially designed ram extruder.

Release of the drug from BODI takes place by two phases-

Initial penetration of tear fluid into the insert inducing a high release rate of drug by diffusion and forming a gel layer around the core of the insert.

The external gelification induces the second period, which corresponds to a slower release rate, but which is still controlled by a diffusion mechanism.

F. Corneal collagen shields:

Collagen is a structural protein than can be applied safely to the body for medical purpose. The creation

of collagen shield has provided a means to promote wound healing and perhaps more importantly to deliver a variety of modifications to the cornea and other ocular tissues.
The preparation of collagen shields includes extraction of the collagen and moulding of collagen into a contact lens configuration. The shields are 14.5 mm in diameter with a 9 mm base curve and thickness of 0.15-0.19 mm. The shields are sterilized by gamma irradiation then dehydrated and individually packaged for storage.

Drugs can be incorporated into collagen matrix during manufacture absorbed into the shields in the eye. As the shield dissolves the drug is released gradually in the tear film and into the aqueous humor (Menqi, 2004). The simplicity of use and the convenience afforded by shields make them an attractive delivery device.

G. Implants:

Scleral and vitreal implants are developed for treatment of endophthalmitis. Implants made from poly lactide/glycolide polymers can result in drug release over 5-6 month period without the need for replacement.

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PULMONARY DRUG DELIVERY SYSTEM

Kamble Pranay, Suryavanshi Kiran, Shaikh Aamir

INTRODUCTION:

Pulmonary drug delivery system is a needle free technique. The origin of inhaled therapies seen in back 4000 years ago to India, where people smoked the leaves of the Atropa belladonna plant to suppress cough. In the 19th and early 20th centuries, asthmatics smoked asthma cigarettes that contained stramonium powder mixed with tobacco to treat the symptoms of their disease. But administration of drugs by the pulmonary route is technically challenging because oral deposition can be high, and variation in inhalation techniques can affect the quantity of a drug delivered to the lungs. Pulmonary drug delivery remains the preferred route for administration of various drugs. It is an important research area which impacts the treatment of illnesses including asthma, chronic obstructive pulmonary disease and various diseases. Due advancement in application nowadays Pulmonary drug delivery is useful to treat Diabetes, angina pectoris, cancer , bone disorders , migraine, tuberculosis, acute lung injury and others.

An inhalation therapy that is effective and safe depends not only on a pharmacologically active molecule, but also on a delivery system and its application. The respiratory tract is exposed to a relatively large number of biological and non biological particulates. These are contained in the 20,000 L of air that must be inhaled daily to accomplish gas exchange. It is a characteristic of the effectiveness of lung defense mechanisms that in healthy people’s lungs are sterile below the larynx. By pulmonary route drug goes gives direct to a target organ1. In the treatment of obstructive respiratory diseases, pulmonary delivery can minimize systemic side effects, provide rapid response and minimize the required dose since the pulmonary route is better alternative to other routes because if we compare it with parental route injection is associated with pain. Secondly, chronic injection is an unpleasant prospect with a host of hygiene issues and potential side effects. Drug delivery by Nasal route is inefficient in terms of the amount of drug actually delivered to the body and to improve its efficiency penetration enhancers must be added that may cause local irritation. But in case of pulmonary route research has shown that many molecules are absorbed through the deep lung into the bloodstream naturally with relatively high bioavailability and without the need for enhancers used by other noninvasive routes. Intra dermal route offers an even less naturally permeable boundary to macromolecules than the gastrointestinal tract. The devices, which inject proteins like insulin, have been available for years, however they have not very well accepted by doctors as well as patients due to the discomfort and the potential for ‘‘splash back’’ to transmit blood borne diseases associated with it .Thus Pulmonary route is better route as compare to other routes. The dosage forms used for this purpose are known as AEROSOLS. They are the dosage forms containing therapeutically active ingredients that are packaged under pressure in a sealed container and are released as a fine mist of spray upon activation of a suitable valve system. (Nimesh P. Patel* n.d.)

ANATOMY AND PHYSIOLOGY OF LUNGS:

1) Lung regions:-

The respiratory tract starts at the nose and terminates deep in the lung at an alveolar sac. There are a number of schemes for categorizing the various regions of the respiratory tract.

2) Nasopharyngeal region:-

This is also referred to as the “upper airways”, which involves the respiratory airways from the nose down to the larynx.

3) Tracheo-bronchial region:-

This is also referred to as the “central” or “conducting airways”, which starts at the larynx and extends via the trachea, bronchi, and bronchioles and ends at the terminal bronchioles.

4) Alveolar region:-

This is also referred to as the "respiratory airways", "peripheral airways" or "pulmonary region", Comprising the respiratory bronchioles, alveolar ducts and alveoli.

Pulmonary epithelium:-

The lung contains more than 40 different cell types, of which more than six line the airways. The diversity of pulmonary epithelia can be illustrated by examining its structure at three principal levels. (Chaudhari Hiralal S1. 2012)

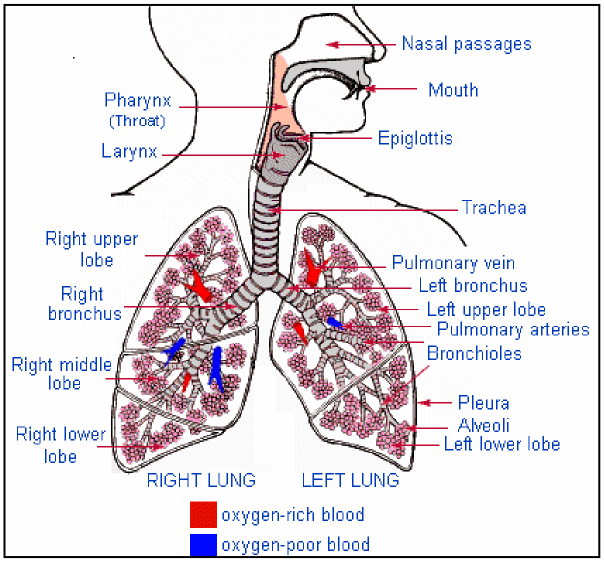

Fig 1: Different regions of the human respiratory tract, (Chaudhari Hiralal S1. 2012)

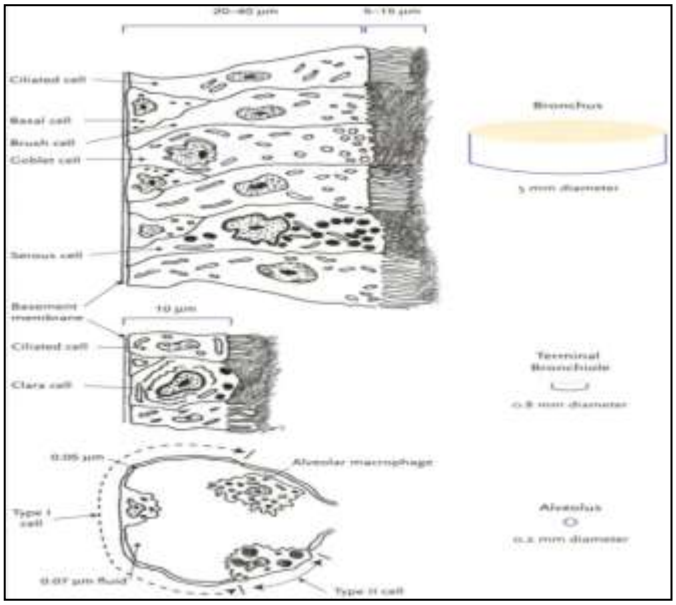

Fig 2: Pulmonary Epithelium, (Chaudhari Hiralal S1. 2012)

The bronchi:-

These are lined predominantly with ciliated and goblet cells. Some serous cells, brush cells and Clara cells are also present with few Kulchitsky cells.

The bronchioles:-

These are primarily lined with ciliated cuboidal cells. The frequency of goblet and serous cells decreases with progression along the airways while the number of Clara cells increases. (Chaudhari Hiralal S1. 2012)

The alveolar region:-

This is devoid of mucus and has a much flatter epithelium, which becomes the simple squamous type, 0.1–0.5 μm thick. Two principal epithelial cell types are present:

Type-I pneumocytes:

Thin cells offering a very short airways-blood path length for the diffusion of gases and drug molecules. Type-I pneumocytes occupy about 93% of the surface area of the alveolar sacs, despite being only half as abundant as type-II cells. (Chaudhari Hiralal S1. 2012)

Type-II pneumocytes:

Cuboidal cells that store and secrete pulmonary surfactant.

Alveolar macrophages account for ~ 3% of cells in the alveolar region. These phagocytic cells scavenge and transport particulate matter to the lymph nodes and the mucociliary escalator. (Chaudhari Hiralal S1. 2012)

Ciliated cells:-

In the trachea bronchial region, a high proportion of the epithelial cells are ciliated such that there is a near complete covering of the central airways by cilia. Towards the periphery of the tracheobronchial region, the cilia are less abundant and are absent in the alveolar region. The ciliated cells each have about 200 cilia with numerous interspersed microvilli, of about 1–2 μm in length. The cilia are hair-like projections about 0.25 μm in diameter and 5 μm in length. They are submersed in an epithelial lining fluid, secreted mainly from the serous cells in the sub-mucosal glands. The tips of the cilia project through the epithelial lining fluid into a layer of mucus secreted from goblet cells. The cilia beat in an planned fashion to propel mucus along the airways to the throat.
(Chaudhari Hiralal S1. 2012)

ADVANTAGES OF PULMONARY DRUG DELIVERY:

1) It is needle free pulmonary delivery.
2) It requires low and fraction of oral dose.
3) Pulmonary drug delivery having very negligible side effects since rest of body are not exposed to drug.
4) Onset of action is very quick with pulmonary drug delivery.
5) Degradation of drug by liver is avoided in pulmonary drug delivery (Karhale Ashish . Jan-March 2012)

DISADVANTAGES OF PULMONARY DRUG DELIVERY:

1) Deposition at oropharyngeal region gives local side effect.
2) Patient may have difficulty using the pulmonary drug devices correctly
3) Drug absorption may be limited by the physical barrier of the mucus layer.
4) Various factors affect the reproducibility on drug delivery on the lungs, including physiological and pharmaceutical barrier.
5) The lungs are not only accessible surface for drug delivery complex but also delivery devices are required to target drug delivery. (Karhale Ashish . Jan-March 2012)

BASIC COMPONENT OF AEROSOL SYSTEM:

- Containers
- Propellants
- Valves
- Actuators

Containers:

Aerosol containers are usually made up of glass, plastic, metal or combination of these materials. Glass containers must be precisely engineered to provide maximum in pressure, safety and impact resistance. Plastics must be employed to coat the glass to improve safety characteristics or to coat metal containers to improve corrosion resistance and enhances the stability of formulation. Suitable metals include stainless steel, aluminum and tin-plated steel. Aerosol containers are made of metal (stainless steel, aluminum or tin-plated steel), glass or plastic or a combination of these materials. The containers must be so designed that they provide the maximum in pressure safety and impact resistance. (Nimesh P. Patel* n.d.)

Propellants:

For pressurized metered dose inhalations propellants perform the essential function of expelling the material from the container by supplying the necessary pressure within the aerosol system. They are liquefied or compounded gases having vapor pressures exceeding employed to obtain the necessary delivery and spray characteristics of the aerosol. (Nimesh P. Patel* n.d.)

Valves:

The valve regulates the flow of the active ingredient(s) and propellant from the container and determines the spray characteristics of the aerosol. It must be manufactured from materials which are inert to the contents of the aerosol. The commonly used materials are rubber, plastic, aluminum and stainless steel. Products for oral or nasal inhalation require metered-dose valves which ensure delivery of a uniform quantity of spray and an accurate dose of the active ingredient(s), both within specified tolerances, with each activation of the valve. (Nimesh P. Patel* n.d.)

Actuators:

The actuator or adaptor which is fitted to the aerosol valve stem is a device which on depression or any other required movement opens the valve and directs the spray to the desired area. The design of the actuator which incorporates an orifice of varying size and shape and expansion chamber is very important in influencing the physical characteristics of the spray or foam, particularly in the case of inhalation aerosols, where the active ingredient(s) must be delivered in the proper particle size range. A proportion of the active ingredient(s) is usually deposited on the inner surface of the actuator; the amount available is therefore less than the amount released by actuation of the valve. (Nimesh P. Patel* n.d.)

INHALERS USED IN PULMONARY DRUG DELIVERY SYSTEM:

Metered dose inhalers:

Metered dose inhalers are the most commonly used devices for generation of aerosol. The drug is micronized and is dispersed in propellant. Surfactants are added to prevent clumping. Upon actuation the propellant gets exposed to atmospheric pressure, which causes aerosolisation of the drug. As it travels through the air, the aerosol warms up, leading to evaporation of the propellant that reduces the particle size to the desirable range. The fraction of drug to the airways ranges from 5 percent to 15 percent.

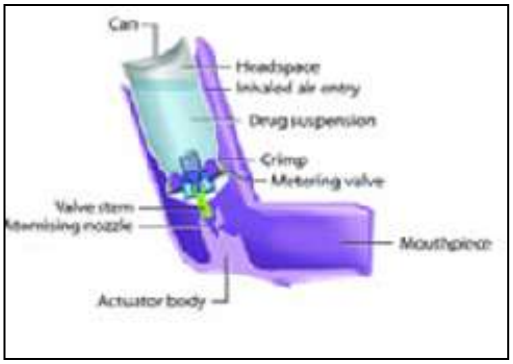

Fig. no. 3- Metered Dose Inhalers (Pierre Carlotti n.d.)

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2) Dry Powdered Inhalers:

Most DPIs contain micronized drugs blended with carrier particles, which prevents aggregation and helps flow. To generate the aerosol, the particles have to be moved. . When the patient activates the DPI and inhales, airflow through the device creates shear and turbulence; air is introduced into the powder bed and the static powder blend is fluidized and enters the patient's airways. There, the drug particles separate from the carrier particles and are carried deep into the lungs, while the larger carrier particles impact in the oropharynx and are cleared. Thus, deposition into the lungs is determined by the patient's variable inspiratory airflow. Dose uniformity is a challenge in the performance of DPIs. This is a greater concern with powders than with liquids because of the size and discrete nature of the particulates. (Nimesh P. Patel* n.d.)

Fig. no.-4: Dry Powdered Inhalers (Nimesh P. Patel* n.d.)

RECENT ADVANCES IN PULMONARY DRUG DELIVERY DEVICES:

Following types of inhalation devices are present Inhalation drug delivery system by- metered dose inhalers Inhalation drug delivery system by — dry powder inhalers Inhalation drug delivery system by -nebulizer.

A) Inhalation drug delivery system by metered dose inhalers:

A metered-dose inhaler (MDI) is a complex system designed to provide a fine mist of medicament, generally with an aerodynamic particle size of less than 5 microns, for inhalation directly to the airways for the treatment of respiratory diseases such as asthma and COPD.

Advances in MDI Technology and Use Enantiomer Preparations of Inhaled Drugs

There has been much interest in the differences in effects of Enantiomer of many medications, and beta agonist adrenergic bronchodilators have received much attention. Recently levo salbutamol active enantiomer of salbutamol is present in market which is free from termers and palpitation that seen in salbutamol. Similarly that the (R) enantiomer of albuterol is mainly responsible for bronchodilator while the (S)-enantiomer may stimulate airway reactivity. Data suggest, however, that after aerosol delivery, the systemic absorption for (R)-albuterol is faster than for (S)-albuterol and that, conversely, the lung retention of (S)-albuterol is longer, which may be detrimental.

Generic proliferation of devices and medications

Nowadays new MDI and nebulizer brands are introduced regularly in pharma market. Even for those who watch this field, it is not unusual to hear a new, unfamiliar brand name regularly. One trend has been the move to generic MDIs and to over the counter availability. These are introduced in the literature by comparing them with well-known older devices. Often, documentation that generic brands or new devices are comparable to older ones is difficult to come by, so comparisons showing pharmacokinetic equivalency are useful.

New technologies to improve patient’s inhalation coordination with MDI:

Spacers are used to improve patient coordination with MDI. Both adults and children often have difficulty coordinating the inhalation effort with the timing of the aerosol puff. Evidence indicates considerable intra and intersubject variabity for the inhalation technique.

Flow gate valve technology in spacers

Certain company s spacer present in market which is static free with valve mechanism which increases drug dose reaching to lungs. Valves opens during inhalation and closed during exhalation this ensures that the residual dose is retained in spacer for subsequent inhalation.

The Auto haler modified form of pMDI

The Auto haler TM is the first breath actuated or activated pressurized metered dose inhaler. Autoihaler solve the key problem of the pressurized metered dose inhaler (pMDI) viz. coordination of actuation with inhalation and does not rely on the patient's inspiratory effort to aerosolize the dose of medication unlike dry powder inhalers .Auto haler is modified form of pressurized metered dose inhaler.

B) Inhalation drug delivery device by dry powder inhalers

Today there are essentially two types of DPIs, those that use drug filled into discrete individual doses, e.g., either a gelatin capsule or a foil–foil blister, and those that use a reservoir of drug that meters out doses when required. Both are now widely available around the globe and are gaining broad acceptance. Unit-Dose -Devices Single-dose powder inhalers are devices in which a powder containing capsule is placed in a holder. The capsule is opened within the device and the powder is inhaled. The capsule residue must be discarded after use and a new capsule inserted for the next dose Multidose Devices - Multidose device uses a circular disk that contains either four or eight powder doses on a single disk. This typically would be treatment for one to two days. The doses are maintained in separate aluminum blister reservoirs until just before inspiration. This device is a true multidose device, having 60 doses in a foil–foil aluminum strip that is opened only at the point just prior to patient inspiration.

BIOLOGICAL MODELS FOR ASSESSMENT OF PULMONARY DRUG ABSORPTION:

Several models are available for preclinical investigations of pulmonary drug absorption and deposition. The complexity of the models range from permeability screening experiments in cell culture models to in vivo pharmacokinetic analyses in animals. The design of the experiments comprises both selection of the most relevant biological model for the specific issue, and the selection of a drug delivery system that is appropriate for the amount of test material available and that can selectively deposit a defined dose of the drug to the intended lung region. A combination of in vitro and in vivo models is needed to elucidate the mechanisms, rate, and extent of absorption, as well as the distribution, metabolism and elimination of a drug after pulmonary administration.
(SIRAJ SHAIKH*1 2010)

In vivo animal models

In vivo pharmacokinetic experiments in animals provide data on the fate of a drug and its metabolites in the body by assessment of the drug concentration in plasma or tissues. Drug exiting device Inhaled dose Systemic exposure Alveolar deposition Pulmonary deposition Tracheal / Bronchial deposition First-pass metabolism / Not absorbed from GI-tract Extrapulmonary deposition Exhaled dose Distribution / Metabolism / Elimination Absorbed AM MC.
AM: phagocytosis by alveolar macrophages
MC: mucociliary clearance Metabolism

In the absence of a significant amount of human absorption data, accurate in vivo pharmacokinetic investigations in animals are important to establish in vitro-in vivo relationships. For determination of the pulmonary absorption rate and bioavailability, plasma is sampled at predetermined time points after pulmonary drug administration and analyzed for drug content (Adjei et al., 1992; Krondahl et al., 2002; Taljanski et al., 1997). An intravenous dose may be administered as reference. For investigations of the retention of the drug in the lung tissue, or first-pass pulmonary uptake, or both, drug concentrations in lung tissue are assessed (Brown et al., 1983; Drew et al., 1981; Jendbro et al., 2001). The applied pulmonary administration procedure should be carefully selected to deliver the dose with high precision regarding both dose quantity and deposition pattern. A disadvantage of the in vivo models is that the animals often need to be anesthetized during drug administration to the lungs and at blood sampling. The effect of anesthesia on physiological functions should thus be considered in the design of the experiments. For instance, the use of volatile anesthetics has been demonstrated to increase the alveolar epithelial permeability (ChangLai et al., 1999; Wollmer et al., 1990) and to destabilize surfactant (Evander et al., 1987; Wollmer et al., 1990). Anesthesia may also impair the mucociliary clearance (Patrick et al., 1977). (Tronde 2002)

Isolated and perfused lungs:

By the use of isolated and perfused lung models, lung-specific pharmacokinetic events can be investigated without the contribution of systemic distribution, metabolism, and elimination. In these models, the structural and cellular integrity of the lung tissue, the permeability barriers, interaction between different cell types, and biochemical activity are maintained (Mehendale et al., 1981). Procedures for lung perfusion have been described for rats, guinea pigs (Ryrfeldt et al., 1978), and rabbits (Anderson et al., 1974). Compared to in vivo models, the isolated and perfused lung models provides certain advantages, such as careful control of the ventilation and perfusion of the lung, facilitated administration of drugs to the airway lumen or vascular circulation, easy sampling of perfusate and lavage fluid, as well as easy determination of mass-balance. Hence, the design of the experiments can be adapted to specifically address issues regarding absorption, tissue sequestration, and metabolism. The main drawback of the lung models is that the limited viability of the preparation (about 5 hours) (Bassett et al., 1992; Fisher et al., 1980) prevents investigations of slow pharmacokinetic processes. Isolated and perfused lung models have successfully been applied to investigate drug dissolution and absorption (Niven et al., 1988; Tronde et al., 2002), mechanisms of absorption (Sakagami et al., 2002a), disposition (Audi et al., 1998; Ryrfeldt et al., 1989), and metabolism (Dollery et al., 1976; Gillespie et al., 1985; Longmore, 1982; Tronde et al., 2002).

Cell culture models

The inaccessibility and heterogeneous composition of the airway epithelium makes it difficult to mechanistically evaluate pulmonary cellular integrity and physiological functions. For investigations of drug transport mechanisms, precise dosing and sampling, as well as defined local drug concentration and surface area of exposure, are important parameters that need to be controllable and reproducible. Therefore, a variety of airway and alveolar epithelial cell culture models of animal and human origin have been established as in vitro absorption models (Elbert et al., 1999; Foster et al., 2000; Morimoto et al., 1993; Winton et al., 1998; Yamashita et al., 1996). The models include both cell lines (airway) and primary cell cultures (airway and alveolar). The primary cell cultures more closely resemble the native epithelia, but are less reproducible and more time-consuming to work with compared to the cell lines, which make them less suitable for permeability screening purposes. Two immortalized human bronchial epithelial cell lines, the Calu-3 and 16HBE14o-, have been suggested as suitable models to investigate the airway epithelial barrier function (i.e., tight junction properties) (Wan et al., 2000; Winton et al., 1998). The Calu-3, adenocarcinoma epithelial cells of serous origin from the bronchial airways, comprise a mixed phenotype of ciliated and secretory cells (Mathias et al., 2002) and form tight, polarized and well differentiated cell monolayers with apical microvilli in air-liquid interface culture (Foster et al., 2000; Mathias et al.,2002). The cell line has recently been applied in some experiments investigating airway drug transport mechanisms (Borchard et al., 2002; Florea et al., 2001; Hamilton et al., 2001a; Hamilton et al., 2001b; Mathias et al., 2002; Pezron et al., 2002). At present there is, to our knowledge, no characterized epithelial cell line available for investigations of the alveolar barrier functions. Instead, alveolar type II cells, isolated from normal human lungs, rats and rabbits, in primary cultures have been demonstrated to differentiate into type-I-like cells and to form tight epithelial barriers morphologically similar to the in vivo alveolar epithelium (Elbert et al., 1999; Matsukawa et al., 1997; Shen et al., 1997). These models have been used for several investigations of alveolar transport (Dodoo A.N. et al., 2000a; Elbert et al., 1999; Matsukawa et al., 1996; Morimoto et al., 1993; Saha et al., 1994; Shen et al., 1997). (Tronde 2002)

FACTORS WHICH AFEECT ABSORPTION RATE AND BIOAVAILABILITY OF PULMONARY DDS:

Device and Formulation	Drug	Physiology
Particle properties (size,density,shape,charge)	Dissolution rate, Solubility	Breathing pattern Blood flow
Deposition pattern	Lipophilicity	Airway morphology
Excipients	molecular weight	Surface area
Concentration	Charge	Mucociliary clearance
Osmolarity	Hydrogen bonding potential	Lung surfactant
Viscosity	Aggregation/complex binding	Alveolar macrophages
pH	Conformation	Epithelial permeability
Dose size/volume	Chemical stability	Endothelial permeability
	Enzymatic stability	Enzymatic/metabolic activity
		Disease
		Tissue composition

Table no.2: Factors that may affect pulmonary absorption rate and bioavailability.
(Tronde 2002)

RECENT APPLICATIONS OF PULMONARY DRUG DELIVERY:

Apart from asthma and COPD recently pulmonary drug delivery is used for following indication Insulin by Aerosol

1. Nicotine Aerosol for Smoking Cessation
2. Aerosols for Angina.
3. Aerosol Vaccination.
4. Aerosols in Transplantation
5. Pulmonary arterial hypertension
6. Treatment of Migraine
7. Acute Lung Injury
8. Gene Therapy via Aerosol
9. In Cancer chemotherapy
10. Amphotericin B
11. Gentamycin aerosol
12. Ribavirin Aerosol
13. Zanimivir R/C with revolizer for swine flue.
14. Inhaled Drug Delivery for Tuberculosis Therapy
15. Pulmonary delivery of lower molecular weight Heparin.
16. Controlled delivery of drugs to lungs
17. Pulmonary delivery of drugs for bone disorders
18. Pulmonary delivery of opioids as pain therapeutics.

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NANOPHARMACEUTICALS

Kulkarni Akshada, Patel Ruchita, Mogal Rajendra

Nanotechnology is the art of manipulating matter at the nanometer scale to create novel structures, devices, and systems. Nanometer scale range = 1 nm to 1000 nm. Reducing the size of a bulk material to nanometer size changes its properties get drastically. Changed properties can be used to develop new technologies. Nanopharmaceuticals are the pharmaceuticals designed by using "nanotechnology ". Drug particles in the nanometer size range have unique characteristics that can lead to enhanced performance in a variety of dosage forms. Nanopharmaceuticals are selected for characteristics such as biodegradability, biocompatibility, conjugation, complexation or encapsulation and their ability to be functionalised. (Prasanna L 2010)

Various formulation parameters that play a crucial role in the successful formulation of drugs are aqueous solubility, stability at ambient temperature and humidity, photo stability, compatibility with solvent and excipient. Among this aqueous solubility became a hurdle for the formulation of new molecular entities. More than 40% of the new chemical entities being generated through drug discovery programmes are poorly water-soluble or lipophilic compounds. Formulating a poorly water soluble drug has always been a challenging problem confronted by the pharmaceutical scientist. (Prasanna L 2010)

The formulation of nano-sized particles can be implemented to all drug compounds belonging to biopharmaceutical classification system (BCS) classes II and IV to increase their solubility and hence partition into gastrointestinal barrier2. Micronization is used for class II drugs of (BCS), i.e. drugs having a good permeability and poor solubility. There are many conventional methods for increasing the solubility of poorly soluble drugs, which include micronization, solubilisation using co-solvents, salt form, surfactant dispersions, precipitation technique, and oily solution. Other techniques are like liposomes, emulsions, microemulsion, solid dispersion and inclusion complexation using cyclodextrins. (Prasanna L 2010)

Nanotechnology can be used to solve the problems associated with these conventional approaches for solubility and bioavailability enhancement. Nanosuspension is favoured for compounds that are insoluble in water (but are soluble in oil) with high log P value, high melting point and high doses. Nanosuspension technology can also be used for drugs which are insoluble in both water and organic solvents. Hydrophobic drugs such as naproxen22, clofazomine23, bupravaquone24, nimesulide25, mitotane26, amphotericin B27, omeprazole28, nifedipine29 and spironolactone30 are formulated as nanosuspension. (Prasanna L 2010), (Bawa R 2008)

Nanopharmaceuticals are devided in two types:

(1) Nanoengineered products and

(2) Nanocarriers

(1) NANOENGINEERED PRODUCTS:

Nanoengineered products includes **nanoparticles, nanosuspension, Nanoemulsion, nanogels,** etc.These are the pharmaceutical dosage forms which contain nanosystem for their application. These products are desigened by converting simple dosage forms into nanosystem by using engineering techniques. This nano -designed products help in: Increase in the solubility and bioavailability of drug molecules. Development of different dosage forms (Ex. Nano-suspensions for parentrals).Exploring different routes of administrations (Ex. Nasal and ophthalmic delivery provides a patient-friendly alternative). (B n.d.)

NANOSUSPENSIONS

Pharmaceutical nanosuspensions are usually very finely dispersed solid drug particles in an aqueous vehicle for either oral and topical use or for parenteral and pulmonary administration. The key difference from conventional suspensions is that the particle size distribution of the solid particles in nanosuspensions is usually less than 1 μm, with an average particle size range between 200 and 600 nm. (Fattal E n.d.)

A nanosuspension not only solves the problem of poor solubility and bioavailability but also alters the pharmacokinetics of drug and that improves drug safety and efficacy. In nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased dissolution rate and therefore improved bioavailability 11.Drugs encapsulated within nanosuspensions exist in pharmaceutically acceptable crystalline or amorphous state. Nanosuspensions can successfully formulate the brick dust molecules for improved dissolution and good absorption. Apart from this, nanosuspensions have some following advantages: firstly, drugs no longer need to be in the soluble form. It is effective for those molecules insoluble in oils ; secondly, the high drug loading can be achieved as a drug exists in the form of pure solids, and can significantly reduce the administration volume of high dose ; thirdly, nanosuspensions can increase the physical and chemical stability of drugs as they are actually in the solid state ; finally, nanosuspensions can provide the passive targeting. (C Prabhakar 2011)

Preparation Methods Of Nanosuspensions

There are two methods for preparation of nanosuspension. They are 'Bottom up technology' and 'Top down technology'. For the production of nanoparticles in Bottom up technology the drug is dissolved in a solvent, which is then added to non-solvent that causes precipitation of the fine drug particles. The top down technologies include-
(a)Media milling
(b)High pressure homogenization
(c)Emulsion solvent diffusion method
(d)Supercritical fluid method and these are preferred over the precipitation methods. (Prasanna L 2010)

Media milling-

The milling process is performed under controlled temperatures. The high energy and shear forces generated as a result of the impaction of the milling media with the drug provide the energy input to break the microparticulate drug into nano-sized particles. The unimodal distribution profile and mean diameter of <200, require a time profile of 30-60 min. The media milling procedure can successfully process micronized and non-micronized drug crystals. Once the formulation and the process are optimized, very short batch-to-batch variation is observed in the quality of the dispersion. A nanosuspension of Naproxen with a mean particle size of 300-600 nm was prepared using pearl milling technique. (Prasanna L 2010)

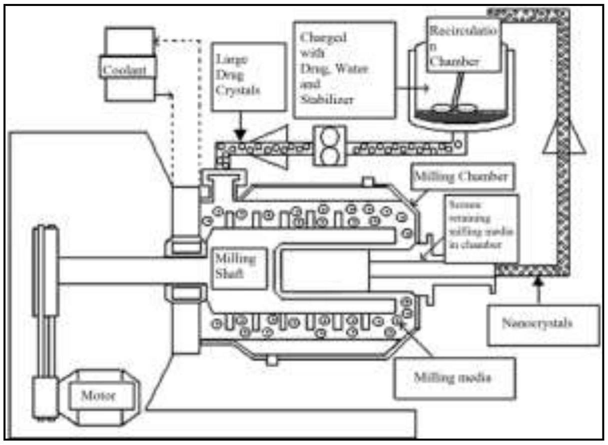

Fig1: Milling method (Patravale V B 2004)

Principle-

The high energy and shear forces generated as a result of the impaction of the milling media with the drug provide the energy input to break the microparticulate drug into nano-sized particles. The milling medium is composed of glass, zirconium oxide or highly cross-linked polystyrene resin. The process can be performed in either batch or recirculation mode. In batch mode, the time required to obtain dispersions with unimodal distribution profiles and mean diameters<200nm is 30–60 min. The media milling process can successfully process micronized and non-micronized drug crystals. Once the formulation and the process are optimized, very little batch-to-batch variation is observed in the quality of the dispersion. (Patravale V B 2004)

Advantages (Patravale V B 2004)

- Drugs that are poorly soluble in both aqueous and organic media can be easily formulated into nanosuspensions.
- Ease of scale-up and little batch-to-batch variation.
- Narrow size distribution of the final nano-sized product. A comparison of the size of naproxen crystals before and after media milling is given in

- Flexibility in handling the drug quantity, ranging from 1 to 400mgmL^{-1}, enabling formulation of very dilute as well as highly concentrated nanosuspensions.

Disadvantages (Patravale V B 2004)

- The major concern is the generation of residues of milling media, which may be introduced in the final product as a result of erosion. This could be problematic when nanosuspensions are intended to be administered for a chronic therapy. The severity of this problem has been reduced to a great extent with the advent of polystyrene resin-based milling medium. For this medium, residual monomers are typically 50 ppb and the residuals generated during the milling processing are not more than 0.005% w/w of the final product or the resulting solid dosage form.

High pressure homogenization-

Drug powders are dispersed in a stabilizer solution to form presuspension; then pre-suspension was homogenized by the highpressure homogenizer at a low pressure for several times as a kind of premilling, and finally was homogenized at a high pressure for 10-25 cycles until the nanosuspensions with the desired size were prepared. (C Prabhakar 2011)

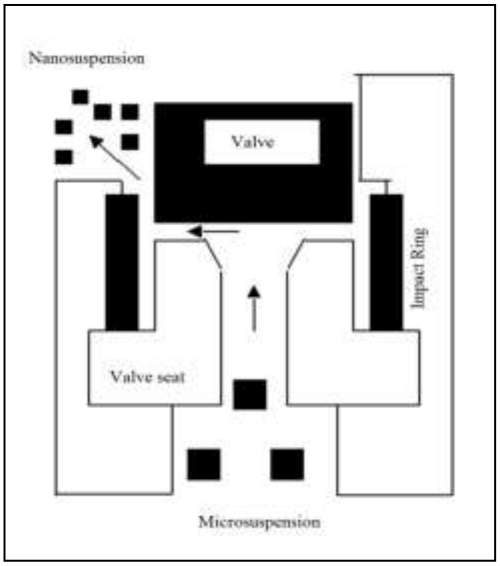

Fig2: Schematic representation of the high-pressure homogenization process (Patravale V B 2004)

Principle-

During homogenization, the fracture of drug particles is brought about by cavitation, high-shear forces and the collision of the particles against each other. The drug suspension, contained in a cylinder of diameter about 3 mm, passes suddenly through a very narrow homogenization gap of 25 µm, which leads to a high streaming velocity. In the homogenization gap, according to Bernoulli's equation, the dynamic pressure of the fluid increases with the simultaneous decrease in static pressure below the boiling point of water at room temperature. In consequence, water starts boiling at room temperature, leading to the formation of gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal air pressure is reached again. The implosion forces are sufficiently high to break down the drug microparticles into nanoparticles. Additionally, the collision of the particles at high speed helps to achieve the nano-sizing of the drug. To improve the efficiency of nano-sizing, the addition of viscosity enhancers is advantageous in certain cases as increasing the viscosity increases the powder density within the dispersion zone (homogenization gap). (Patravale V B 2004)

Advantages (Patravale V B 2004)

- Drugs that are poorly soluble in both aqueous and organic media can be easily formulated into nanosuspensions.
- Ease of scale-up and little batch-to-batch variation.
- Narrow size distribution of the nanoparticulate drug present in the final product.
- Allows aseptic production of nanosuspensions for parenteral administration.
- Flexibility in handling the drug quantity, ranging from 1 to 400mgmL^{-1}, thus enabling formulation of very dilute as well as highly concentrated nanosuspensions

Disadvantages (Patravale V B 2004)

- Prerequisite of micronized drug particles.
- Prerequisite of suspension formation using high-speed mixers before subjecting it to homogenization.

Emulsion solvent diffusion method-

There are two ways of fabricating drug nanosuspensions by the emulsification method. (Patravale V B 2004)

1. An organic solvent or mixture of solvents loaded with the drug is dispersed in the aqueous phase containing suitable surfactants with stirring to form an emulsion. The obtained emulsion was further homogenized by high pressure homogenization. After homogenization cycles the emulsion was diluted with water, homogenized by homogenizer to diffuse the organic solvent and convert the droplets into solid particles. Since one particle is formed in each emulsion droplet, it is possible to control the particle size of the nanosuspension by controlling the size of the emulsion. (Prasanna L 2010)

Advantages (Patravale V B 2004)

- Use of specialized equipment is not necessary.
- Particle size can easily be controlled by controlling the size of the emulsion droplet.
- Ease of scale-up if formulation is optimized properly.

Disadvantages (Patravale V B 2004)

- Drugs that are poorly soluble in both aqueous and organic media cannot be formulated by this technique.
- Safety concerns because of the use of hazardous solvents in the process.
- Need for diultrafiltration for purification of the drug nanosuspension, which may render the process costly.
- High amount of surfactant/stabilizer is required as compared to the production techniques described earlier.

2. Use partially water-miscible solvents such as butyl lactate, benzyl alcohol and triacetin as the dispersed phase instead of hazardous solvents. The emulsion is formed by the conventional method and the drug nanosuspension is obtained by just diluting the emulsion. Dilution of the emulsion with water causes complete diffusion of the internal phase into the external phase, leading to instantaneous formation of a nanosuspension. The nanosuspension thus formed has to be made free of the internal phase and surfactants by means of diultrafiltration in order to make it suitable for administration. (Patravale V B 2004)

Advantages (Patravale V B 2004)

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Disadvantages (Patravale V B 2004)

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Supercritical fluid method-

The organic solvents used in the preparation of conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods are hazardous to environment and physiological systems. To rectify the problem occurred through the conventional method supercritical fluid technology has been investigated for the preparation of biodegradable micro and nanoparticles, because supercritical fluids are environmentally safe. The most common techniques using supercritical fluids are supercritical anti-solvent (SAS), precipitation with compressed anti-solvent process (PCS) , and rapid expansion of supercritical solution (RESS). The process of SAS employs a liquid solvent, e.g. methanol, which is completely miscible with the supercritical fluid (SC CO_2), to dissolve the solute to be micronized; at the process condition, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting in the formation of nanoparticles. Dexamethasone49 phosphate drug nanoparticles (for microencapsulation) and griseofulvin50 nanoparticles were prepared by using SAS method. RESS differs from the SAS process in that its solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is rapidly expanded through a small nozzle into a region lower pressure, thus the solvent power of supercritical fluid dramatically decreases and solute eventually precipitates. This method is used for the production of polymeric nanoparticles51. Cyclosporine nanoparticles52 were prepared by using RESS method. The drug solution is atomized into a chamber containing compressed CO_2 in PCA method. The solution gets supersaturated when the solvent is removed and therefore precipitated as fine crystals. (Prasanna L 2010)

Formulation Consideration: (Prasanna L 2010)

Stabilizer-

The main function of a stabilizer are to wet the drug particles thoroughly, and to prevent Ostwald's ripening and agglomeration of nanosuspension in order to yield a physically stable formulation by providing steric or ionic barrier. The type and amount of stabilize has a pronounced effect on the physical stability and in vivo behaviour of nanosuspension. Stabilizers that have been used so far are poloxomers, polysorbate, cellulosics, povidones, and lecithins. Lecithin is the stabilizer of choice if one intends to develop a parentally acceptable and autoclavable nanosuspension.

Organic solvent-

Organic solvents are used in the formulation of nanosuspension if emulsions or microemulsions are used as a template. The pharmaceutically acceptable less hazardous water miscible solvent,

such as methanol, ethanol, chloroform, ispropanol, and partially water miscible solvents ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate, benzyl alcohol, are preferent in the formulation over the conventional hazardous solvents, such as dichloromethane.

Other additives

Nanosuspensions may contain additives such as buffers, salts, polyols, osmogent and cryoprotectant, depending on either the route of administration or the properties of the drug moiety.

Characterization Of Nanosuspension: (Prasanna L 2010)

Charecterization parameters for nanosuspensions are size and size distribution, particle charge (zeta potential), crystalline status, as well as dissolution velocity and saturation solubility.

Particle size distribution-

The most important charecterization parameter for the nanosuspension are the mean particle size and width of particle size distribution (called polydispersity index) which governs the physicochemical properties like saturation solubility, dissolution velocity, physical stability and even biological performance. Change in particle size changes saturated solubility and dissolution velocity. Different methods for determining particle size distribution are photon correlation spectroscopy (PCS), laser diffraction (LD), and coulter counter multisizer. PCS can even be used for determining the width of the particle size distribution (polydispersity index, PI). The PI is an important parameter that governs the physical stability of nanosuspensions and should be as low as possible for the long-term stability of nanosuspensions. A PI value of 0.1–0.25 indicates a fairly narrow size distribution where as a PI value greater than 0.5 indicates a very broad distribution. PCS determines the particle size in the range of (3nm to 3 μm) it becomes difficult to determine the possibility of contamination of the nanosuspension by microparticulate drugs (having particle size greater than 3μm). Hence, in addition to PCS analysis, laser diffractometry (LD) analysis of nanosuspensions should be carried out in order to detect as well as quantify the drug microparticles that might have been generated during the production process. LD determines the particle size in the range of 0.05-80μm up to 2000μm. The typical LD characterization includes determination of diameter 50% LD and diameter 99% LD values, which indicate that either 50 or 99% of the particles are below the indicated size. For parental use the particle size should be less than 5µm, considering that the smaller size of the capillaries is 5-6µm and hence a higher particle size can lead to capillary blockade and embolism. For nanosuspensions that are intended for intravenous administration, particle size analysis by the Coulter counter technique is essential in addition to PCS and LD analysis. Since the Coulter counter gives the absolute number of particles per volume unit for the different size classes.

Zeta potential (particle charge)-

Zeta potential determines the physical stability of nanosuspenson. Zeta potential is an indirect measurement of the thickness of the diffusion layer, i.e. can be used to predict long term stability53. In order to obtain a nanosuspension exhibiting good stability, for an electrostatically stabilized nanosuspension a minimum zeta potential of ± 30mv is required whereas in the case of a combined electrostatic and steric stabilization, a minimum zeta potential of ± 20mV is desirable.

Crystal morphology-

X-ray diffraction analysis in combination with differential scanning calorimetry, scanning electron microscopy is used to determine the polymorphic changes due to impact of high pressure homogenization in the crystalline structure of the drug. Nanosuspension can undergo a change in the crystalline structure, which may be to an amorphous form or to other polymorphic forms because of high pressure homogenization. An increased amount of amorphous drug fraction could induce higher saturation solubility.

Saturation solubility and dissolution velocity-

Nanosuspension increases the dissolution velocity and saturation solubility. Size reduction leads to increase in the dissolution pressure. An increase in solubility that occurs with relatively low particle size reduction may be mainly due to a change in surface tension leading to increased saturation solubility.

Applications: (C Prabhakar 2011)

Applications of nanosuspensions had land

Marking history and the applications given are few.

Oral drug delivery

The oral route is the preferred route for drug delivery because of its numerous well-known advantages. .Orally administered antibiotics such as atovaquone and bupravaquone reflect this problem very well. Nanosizing of such drugs can lead to a dramatic increase in their oral absorption and subsequently bioavailability. The oral administration of naproxen nanoparticles lead to an area under the curve (AUC) (0-24 h) of 97.5 mg-h/l compared with just 44.7 mg-h/l for naprosyn suspensions and 32.7 mg-h/l for anaprox tablets 35. Oral administration of the gonadotrophin inhibitor Danazol as a nanosuspension leads to an absolute bioavailability of 82.3 and the conventional dispersion (Danocrine) only to 5.2% 36. A nanosuspension of Amphotericin B developed by Kayser et al.showed a significant improvement in its oral absorption in comparison with the conventional commercial formulation.

Parenteral drug delivery

One of the important applications of nanosuspension technology is the formulation of intravenously administered products. IV administration results in several advantages, such as administration of poorly soluble drugs without using a higher concentration of toxic co-solvents, improving the therapeutic effect of the drug available as conventional oral formulations and targeting the drug to macrophages and the pathogenic microorganisms residing in the macrophages. Peters et al. prepared clofazimine nanosuspensions for IV use and showed that the drug concentrations in the liver, spleen and lungs reached a comparably higher level, well in excess of the minimum inhibitory concentration for most Mycobacterium avium strains. Injectable nanosuspensions of poorly soluble drug tarazepide have been prepared to overcome the limited success achieved using conventional solubilization techniques, such as use of surfactants, cyclodextrins, etc., to improve bioavailability.

Pulmonary drug delivery

Nanosuspensions may prove to be an ideal approach for delivering drugs that exhibit poor solubility in pulmonary secretions. Aqueous nanosuspensions can be nebulized using mechanical or ultrasonic nebulizers for lung delivery. Because of their small size, it is likely that in each aerosol droplet at least one drug particle is contained, leading to a more uniform distribution of the drug in lungs. The nanoparticulate nature of the drug allows the rapid diffusion and dissolution of the drug at the site of action. At the same time, the increased adhesiveness of the drug to mucosal surfaces offers a prolonged residence time for the drug at the absorption site. This ability of nanosuspensions to offer quick onset of action initially and then controlled release of the activemoiety is highly beneficial and is required by most pulmonary diseases. Budenoside drug nanoparticles were successfully nebulized using an Ultrasonic nebulizer.

Ocular drug delivery

Nanosuspensions can prove to be a boon for drugs that exhibit poor solubility in lachrymal fluids. Nanosuspensions, by their inherent ability to improve the saturation solubility of the drug, represent an ideal approach for ocular delivery of hydrophobic drugs and Nanoparticulate nature of the drug allows its prolonged residence in the culdesac, giving sustained release of the drug. Pignatello et al. prepared Eudragit retard nanosuspensions of cloricromene for ocular delivery. They observed that the drug showed a higher availability in rabbit aqueous humor. The polymeric nanosuspensions of flurbiprofen and ibuprofen have been successfully formulated using acrylate polymers such as Eudragit RS 100 and Eudragit RL 100. The ocular anti-inflammatory activity of Ibuprofen-Eudragit RS100 nanosuspensions was greatly improved when compared with an aqueous solution of Ibuprofen lysinate. Further, the aqueous humor drug concentration was significantly higher in groups treated with Ibuprofen-Eudragit RS when compared with the Ibuprofen- treated group.2

Targeted drug delivery

Nanosuspensions can be used for targeted delivery as their surface properties and in-vivo behavior can easily be altered by changing either the stabilizer or the milieu. The engineering of stealth nanosuspensions (analogous to stealth liposomes) by using various surface coatings for active or passive targeting of the desired site is the future of targeted drug delivery systems.31Kayser formulated a nanosuspension of Aphidicolin to improve drug targeting against leishmaniainfected macrophages. He stated that the drug in the conventional form had an effective concentration (EC 50) of 0.16 mcg/ml whereas the nanosuspension formulation had an enhanced activity with an EC (50) of 0.003 mcg/ml.42 Scholer et al. showed an improved drug targeting to the brain in the treatment of toxoplasmic encephalitis in a new murine model infected with Toxoplasma gondii using a nanosuspension formulation of Atovaquone.

Mucoadhesion of the Nanoparticles

Nanoparticles orally administered in the form of a suspension diffuse into the liquid media and rapidly encounter the mucosal surface.The direct contact of the particles with the intestinal cells through a bioadhesive phase is the first step before particle absorption. The adhesiveness of the nanosuspensions not only helps to improve bioavailability but also improves targeting of the parasites persisting in the GIT, e.g.,Cryptosporidium parvum.2Mucoadhesive bupravaquone nanosuspensions, because of their prolonged residence at the infection site, revealed a 10-fold reduction in the infectivity score of Cryptosporidium parvum as compared to the bupravaquone nanosuspensions without mucoadhesive polymers.

NANOPARTICLES

Nanoparticles are small colloidal particles which are made of non-biodegradable and biodegradable polymers. Their diameter is generally around 200 nm. Preparation of particles was simple and easy to scale-up and particles formed were stable and easily freeze-dried. Due to these reasons, nanoparticles made of biodegradable polymers were developed for drug delivery. In addition, nanoparticles were able to protect drugs against chemical and enzymatic degradation and were also able to reduce side effects of some active drugs. (Fattal E n.d.)

Production of nanoparticles (Mohanraj V J 2006)

There are a wide variety of techniques that are capable of creating nanostructures with various degrees of quality, speed, and cost . These manufacturing approaches fall under two categories: Bottom-up Technique Top-down Technique.

Bottom up technique- Bottom-up manufacturing involves the building of nanostructures atom by atom or molecule by molecule. This can be done in three ways: chemical synthesis, self assembly, and positional assembly .

Top down technique- Top-down manufacturing involves starting with a larger piece of material, and etching, milling, or machining a nanostructure from it by removing material. Comminuting or grinding or milling is the oldest mechanical unit operation for size reduction of solids and for producing large quantities of particulate materials.

Some production techniques

1) **PEARL/BALL-MILLING TECHNOLOGY-** Milling container filled with fine milling pearls or larger-sized balls . The container can be static and the milling material is moved by a stirrer; alternatively, the complete container is moved in a complex movement leading consequently to movement of the milling pearls . Material used- steel, glass, and zircon oxide Polymer used- hard polystyrene . A problem associated with the pearl milling technology is the erosion from the milling material during the milling process. (Mohanraj V J 2006)

2) HIGH-PRESSURE HOMOGENIZATION- High-pressure homogenization is currently used in the food industry, e.g., homogenization of milk. In the pharmaceutical industry parenteral emulsions are produced by this technology. Typical pressures for the production of drug nanosuspensions are 1000–1500 bar (corresponding to 100–150 Mpa, 14504–21756 psi); the number of required homogenization cycles varies from 10 to 20 depending on the properties of the drug . Most of the homogenizers used are based on the piston-gap principle; an alternative is the Jetstream technology. (Mohanraj V J 2006)

3) SPRAY-DRYING- Two-step process of the production of drug nanocrystal loaded compounds: the drug nanosuspension obtained by high pressure homogenization is further processed by spray drying . Drug nanocrystals embedded in the matrix are obtained. (Mohanraj V J 2006) (Okuyama K 2004)

4)SOL GEL METHOD- Aqueous or alcohol-based Involves use of molecular precursors, mainly alkoxides Alternatively, metal formates Mixture stirred until gel forms Gel is dried at 100 C for 24 hours over a water bath, then ground to a powder Powder heated gradually (5 C/min), calcined in air at 500 – 1200 C for 2 hours Allows mixing of precursors at molecular level better control High purity Low sintering temperature High degree of homogeneity Particularly suited to production of nano -sized multi-component ceramic powders. (Mohanraj V J 2006)

5) MICROFLUIDIZATION- In this technique, suspension of the drug is guided through specialized chambers under high pressure. The chambers consist of narrow openings through which the suspension is forced to pass at high velocities . The chamber geometry divides the suspension into two different streams and these streaming liquid jets are then made to impinge against each other in the impingement area where particle size reduction occurs . Particle size reduction is achieved by high energy impact, cavitation, and shear forces. (Mohanraj V J 2006)

Some other techniques include **Spray pyrolysis, Flame spray pyrolysis, Laser pyrolysis, Spark discharge generation, Inert gas condensation.** (Okuyama K 2004)

NANOEMULSION (B n.d.)

The term “Nanoemulsion" refers to a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules . A Nanoemulsion is considered to be a thermodynamically or kinetically stable liquid dispersion of an oil phase and a water phase, in combination with a surfactant. The dispersed phase typically comprises small particles or droplets, with a size range of 5 nm-200 nm.

Three types of Nanoemulsions are most likely to be formed depending on the composition:

1. Oil in water Nanoemulsions wherein oil droplets are dispersed in the continuous aqueous phase
2. Water in oil Nanoemulsions wherein water droplets are dispersed in the continuous oil phase;

3. Bi-continuous Nanoemulsions wherein microdomains of oil and water are interdispersed within the system.

In all three types of Nanoemulsions, the interface is stabilized by an appropriate combination of surfactants and/or co-surfactants.

Classification of Surfactants-

- Nonionic - Fatty alcohols, glycerol esters, fatty acid esters
- Anionic- Contain carboxylate groups. Soaps, Sulfonates, Divalent ions.
- Cationic- Amines and quaternary ammonium compounds. Cetyl trimethyl ammonium bromide.

Evalution parameter-

1. Dye Solubilization
2. Dilutability Test
3. Conductance Measurement
4. Dynamic light-scattering measurements
5. Polydispersity
6. Interfacial Tension
7. Viscosity measurement
8. In-vitro Drug Permeation Studies
9. Determination of permeability coefficient and flux.

NANOGELS (B n.d.)

Up till now pharmaceutical field have witnessed developed of organogels and hydrogels, the recent addition to this are nanogels. Nanogels are crosslinked particles of sub- micrometer size made of hydrophilic polymers. (Vinogradov S V 2005) They are soluble in water, but have properties different from linear macromolecules of similar molecular weight. These are formed either through covalent bonds for stable and insoluble three-dimensional networks or unstable (physical) gels via hydrogen bonds, van der Waals forces, and chain entanglements or through formation of crystalline regions. (K 2007)

Techniques for preparation of nanogels-

1. Micromolding method
2. Microfluidic preparation
3. Dispersion polymerization
4. Precipitation polymerization
5. Inverse (mini)emulsion polymerization

Application-

1. Mucoadhesive microgels
2. Metal-containing nanocarriers
3. Polymeric nanogels
4. Systemic drug delivery.

(2) NANOCARRIER PRODUCTS

Nanocarriers includes **polymer micelles, dendrimer, liposomes, neosomes, quantam dots, nanotubes**, etc.Nanocarriers are colloidal particulate systems with size ranging between 10-1000 nm. They have been successfully utilized in the diagnosis, treatment and monitoring of various diseases. It was reported that particles less than 200 nm can escape this physical screening, hence, Nanoparticulate carriers might be of great interest. There are various types of nanocarriers are introduced nowadays such as: Polymer micelle Dendrimers Liposomes Quantum dots.

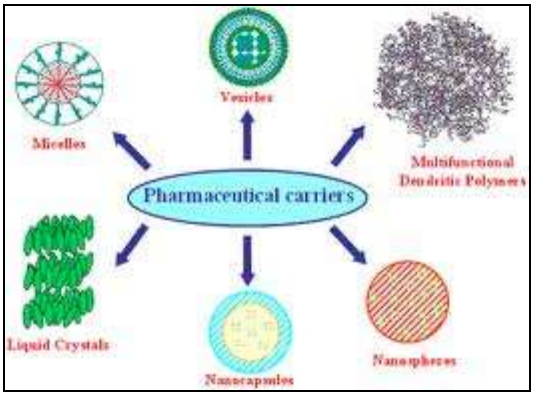

Fig 3: diagram of pharmaceutical carriers.

POLYMER MICELLE

Polymeric micelles are nano -sized particles that are made up of polymer chains and are usually spontaneously formed by self-assembly in a liquid, generally as a result of hydrophobic or ion pair interactions between polymer segments. The core of the micelles, which is either the hydrophobic part or the ionic part of the nanoparticles, can contain small (or bigger) molecules such as therapeutic drugs, while the shell provides interactions with the solvent and make the nanoparticles thereby stable in the liquid.

Depending on the delivery purpose, one can select the size, charge, and surface properties of these carriers simply by adding new ingredients to the mixture of amphiphilic substances before micelle preparation and/or by variation of the preparation method.

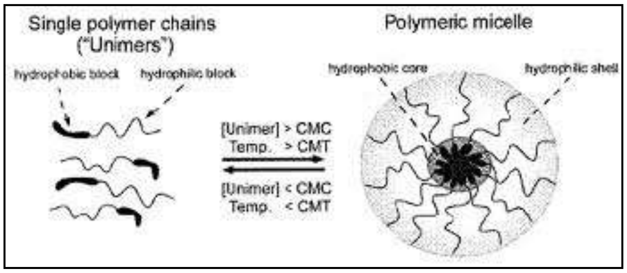

Fig4: self assembled micelles.

Drug loading and release

In general, there are three major methods for loading drugs into polymer micelle cores : (1) chemical conjugation, (2) physical entrapment or solubilization (3) polyionic complexation (e.g. ionic binding).

Application

1. Delivery of anticancer agents to treat tumors.
2. Drug delivery to the brain to treat neurodegenerative diseases.
3. Delivery of antifungal agents.
4. Delivery of imaging agents for diagnostic applications.
5. Delivery of polynucleotide therapeutics.

DENDRIMERS (Marcato P D 2008)

Dendrimer is a highly branched polymer and consists of a core where a monomer unit is attached. Dendrimers are large and complex molecules with very well-defined chemical structures . Dendrimers are built from a starting atom, such as nitrogen, after a repeating series of chemical reactions, carbon and other elements was added into it; produce a spherical branching structure. As the process repeats, result is a spherical macromolecular structure. (Marcato P D 2008)

They consist of three major architectural components: 1. Core 2. Branching Unit 3. End groups

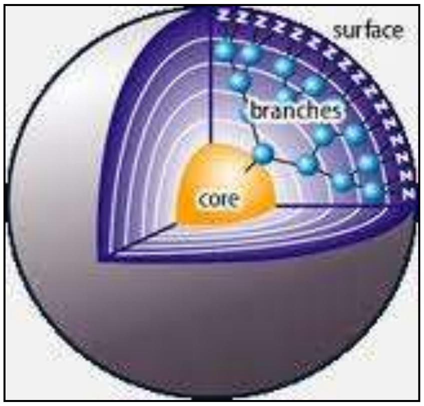

Fig5: dendrimer components.

Synthesis of dendrimer-

1. Divergent growth method
2. Convergent Dendrimer Growth
3. Double Exponential and Mixed Growth
4. Hypercores and Branched Monomers growth.

Applications-

1. Ocular drug delivery
2. Pulmonary drug delivery
3. As bio mimetic artificial proteins
4. As nano –scaffolds

LIPOSOMES (Marcato P D 2008)

Nanoliposomes are nanometric version of liposomes, which are one of the most applied encapsulation and controlled release systems . The word liposome derives from two Greek words, lipos (fat) and soma (body or structure), meaning a structure in which a fatty envelope encapsulates internal aqueous compartment(s). Liposomes are nano size artificial vesicles of spherical shape that can be produced from natural phospholipids and cholesterol.

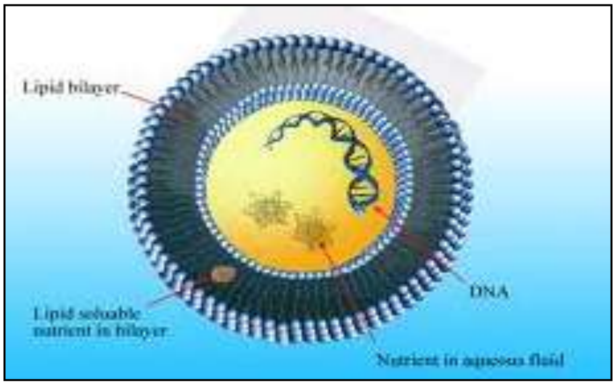

Fig6: liposome as a drug carrier.

Liposomes are broadly classified by their structure Multilamellar liposomes: Spherically concentric multilamellar (many bilayers) structures Unilamellar liposomes: Spherical concentric unilamellar (one bilayer) structures The size, lamellarity (unilamellar or multilamellar) and lipid composition of the bilayers influence many of the important properties like the fluidity, permeability, stability and structure - these can be controlled and customized to serve specific needs.

QUANTUM DOTS (B n.d.)

A major field of research for about the past five years, quantum dots (also called nanocrystals or artificial atoms) represent a special form of spherical nanocrystals from 1 to 10 nm in diameter.

They have been developed in the form of semiconductors, insulators, metals, magnetic materials or metallic oxides. The number of atoms in quantum dots, which can range from 1,000 to 100,000, makes them neither an extended solid structure nor a molecular entity. QDs 10 nm in diameter. (Ostiguy C 2006) QDs can be synthesized from are colloidal semiconductor nanocrystals ranging from 2 to various types of semiconductor materials via colloidal synthesis or electrochemistry. QDs also provide enough surface area to attach therapeutic agents for simultaneous drug delivery. The most commonly used QDs are cadmium selenide (CdSe), cadmium telluride (CdTe), indium phosphide (InP), and indium arsenide (InAs).

SOME OTHER CARRIERS (B n.d.)

Gold Nanoshells: A gold shell surrounding a semiconductor, these can be irradiated when reaching their target. This heats the nanoshell , which, in turn, kills cancer cells.

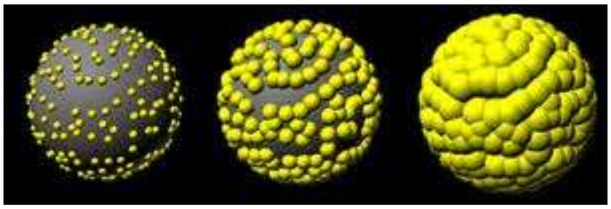

Fig7: gold nanoshells.

Fullerenes: A form of carbon (C-60), fullerenes are composed of carbon atoms arranged in a soccer ball-like shape, hence their other name— buckyballs. Easily manufactured in quantity, they seem to be ideal drug delivery vehicles due to their size and shape.

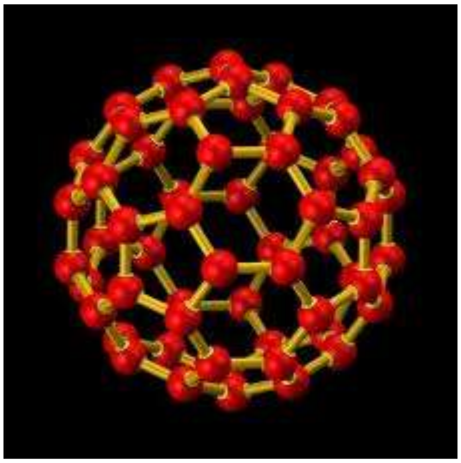

Fig8: diagram of fullerenes.

CARBON NANOTUBES (Marcato P D 2008)

Carbon nanotubes are extremely thin (their diameter is about 10,000 times smaller than a human hair), hollow cylinders made of carbon atoms. TYPES-Single-Walled Carbon Nanotubes (SWCNTs) Multi-Walled Carbon Nanotubes (MWCNTs).

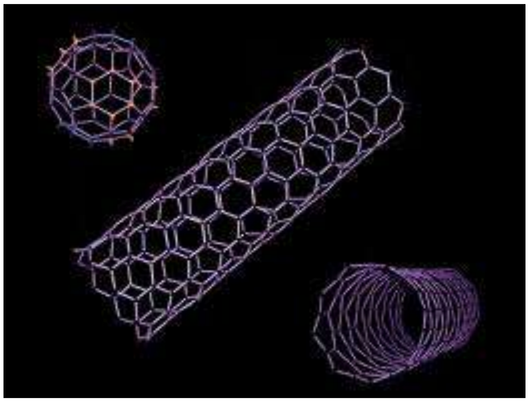

Fig9: carbon Nanotubes.

Advantages of nanopharmaceuticals-

1. Improved Bioavailability
2. Reduced Toxicity
3. Sustained and Controlled Release
4. Ability to Target
5. Do not occlude Blood to Brain and Intracellular Compartment
6. Protects Fragile Drugs/Proteins from Harsh Biological Environment
7. Faster, Safer and More Accurate Disease Diagnosis

Nanopharmaceutical	**Company**	**FDA approval date**
Doxil Caelyx	Pegylated Doxorubicin HCl Liposome	Nov 1995
Abraxane	Paclitaxel bound albumin nanoparticles	Jan 2005
Diprivan	Propofol liposome	Oct 1989
DaunoXome	Encapsulated-Daunorubicin citrate liposome	Apr 1996
Estrasorb	Estradiol hemihydrates micellar nanoparticles	Oct 2003
Macugen	PEG-anti VEGF aptamer	Dec 2004
Amphotec	Colloidal suspension of lipid base Amphoterecin B	Nov 1996

Table1: Examples of Nanopharmaceuticals approved by FDA

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MEDICATED CHEWING GUMS

Basrur Pooja, Katedeshmukh Ramesh

Introduction:

Oral route is the most preferred route amongst the patient and clinicians due to various advantages it offers. Chewing gum as drug delivery system holds tremendous potential not only in smoking cessation and also in oral health areas. Medicated chewing gum is nothing but gum base containing an active substance either in its core or coating. The drug product is intended to be chewed in the oral cavity for a specific period of time, after which the insoluble gum base is discarded. Medicated chewing gums are defined by the European Pharmacopoeia (Ph. Eur.) and the guidelines for pharmaceutical dosage forms issued in 1991 by the Committee for Medicinal Products for Human Use (CPMP) as ‘‘solid single dose preparations with a base consisting mainly of gum that are intended to be chewed but not swallowed, providing a slow steady release of the medicine contained”. It can be taken discreetly without water and the drug is allowed for local and systemic delivery. Chewing gum is one of the modern approaches to oral transmucosal drug delivery and is a useful means for systemic drug delivery. Medicated Chewing Gums (MCG) can also be utilized for site specific activity in case of oral cancer. Dye is used sometimes as a tracer of drug release from medicated chewing gums. (Jayachandar G 2008) (S 2010) (Evelyn O 2008)

Advantages of Medicated chewing Gums:

1. Fast onset due to rapid release of active ingredients in buccal cavity and subsequent absorption in systemic circulation.
2. Avoids First Pass Metabolism and thus increases the bioavailability of drugs
3. Pleasant taste
4. Easy for administration without water promotes
5. Higher patient compliance
6. High acceptances by children and for patients who find swallowing tablets difficult are obvious.
7. A lower dosage of substance may be therapeutically sufficient, possibly resulting in a fewer side effects, and promote fast absorption.
8. Systemic effect or local effect can be achieved
9. Effect on dry mouth (xerostomia)
10. Excellent for acute medication
11. Gum does not reach the stomach. Hence G.I.T. suffers less from the effects of excipients. Stomach does not suffer from direct contact with high concentrations of drug, thus reducing the risk of intolerance of gastric mucosa.
12. Aspirin, Dimenhydrinate and Caffeine shows faster absorption through MCG than tablets. (Heema N 2011) (S 2010) (TUSHAR D 2011)

Optimal Properties of Drug:

Physicochemical Properties of Drug

- High Salivary Solubility
- pH independent solubility
- Tasteless
- Non-toxic to oro-mucosa and salivary ducts
- Non-carcinogenic

Patient Related Factors

- Should not cause tooth decay
- Should not cause oro-mucosa and teeth staining
- Should not affect salivary flow rate (Nilima T 2011)

Overview of oral mucosa:

Structure

The oral mucosa is composed of an outermost layer of stratified squamous epithelium below this lies a basement membrane, a lamina propria followed by the submucosa as the innermost layer. The epithelium of the buccal mucosa is about 40–50 cell layers thick, while that of the sublingual epithelium contains somewhat fewer. The epithelial cells increase in size and become flatter as they travel from the basal layers to the superficial layers.

Permeability

The oral mucosa in general is somewhat leaky epithelia intermediate between that of the epidermis and intestinal mucosa. It is estimated that the permeability of the buccal mucosa is 4–4000 times greater than that of the skin. The permeability of the oral mucosae decrease in the order of, sublingual greater than buccal and buccal greater than palatal. Flattened surface cell layers present are the main barrier to permeation, while the more isodiametric cell layers are relatively permeable.

Environment

The cells of the oral epithelia are surrounded by an intercellular ground substance, mucus, the principle components of which are complexes made up of proteins and carbohydrates. These complexes may be free of association or some may be attached to certain regions on the cell surfaces. This matrix may actually play a role in cell–cell adhesion, as well as act as a lubricant, allowing cells to move relative to one another. In the oral mucosa; mucus is secreted by the major and minor salivary glands as part of saliva. Up to 70% of the total mucin found in saliva is contributed by the minor salivary glands. At physiological pH, the mucus network carries a negative charge (due to the sialic acid and sulfate residues) which may play a role inmucoadhesion. At this pH mucus can form a strongly cohesive gel structure that will bind to the epithelial cell surface as a gelatinous layer. The oral mucosal thickness varies depending on the site: the buccal mucosa measures at 500–800 µm, the mucosal thickness of the hard and soft palates, the floor of the mouth, the ventral tongue, and the gingivae measure at about 100–200 µm. Saliva is the protective fluid for all tissues of the oral cavity. It protects the soft tissues from abrasion by rough materials and from chemicals. It allows for the continuous mineralization of the tooth enamel after eruption and helps in demineralization of the enamel in the early stages of dental caries. Saliva is an aqueous fluid with 1% organic and inorganic materials. The major determinant of the salivary composition is the flow rate which in turn depends upon three factors: the time of day, the type of stimulus. The salivary pH ranges from 5.5 to 7 depending on the flow rate. Main reason behind the selection of hydrophilic polymeric matrices as vehicles for oral trans mucosal drug delivery systems is this water rich environment of the oral cavity.

Absorption from oral mucosa:

The oral cavity comprises the lips, cheek (buccal), tongue, hard palate, soft palate and floor of the mouth. The lining of the oral cavity is referred to as the oral mucosa, and includes the buccal, sublingual, gingival, palatal and labial mucosae. The mucosal tissues in the cheeks (buccal), the floor of the mouth (sublingual) and the ventral surface of the tongue account for about 60% of the oral mucosal surface area. The buccal and sublingual tissues are the primary focus for drug delivery via the oral mucosa because they are more permeable than the tissues in other regions of the mouth. The surface area of the oral mucosa (200 cm^2) is relatively small compared with the gastrointestinal tract (350000 cm^2) and skin (20000 cm^2). However, the oral mucosa is highly vascularized, and therefore any drug diffusing into the oral mucosa membranes has direct access to the systemic circulation via capillaries and venous drainage. Thus, drugs that are absorbed through the oral mucosa directly enter the systemic circulation, bypassing the gastrointestinal tract and first-pass metabolism in the liver. The rate of blood flow through the oral mucosa is substantial, and is generally not considered to be the rate-limiting factor .in the absorption of drugs by this route. In order for a drug to pass through the oral mucosa, it must first diffuse through the lipophilic cell membrane, and then pass through the hydrophilic interior of the cells of the oral epithelium. Thus, the oral mucosa provides both hydrophilic and hydrophobic barriers that must be overcome for efficient mucosal delivery. (Satheesh M 2009)

Formulation of Medicated Chewing Gum:

Some important formulation aspect:

1. Increased amount of softners and emulsifiers in gum base fasten release whereas hard gum may retard.
2. Cyclodextrin complexation or solubilisation technique increases aqueous solubility of drugs that are poorly water soluble.
3. A solid system of lipophilic active ingredients bound to the cation exchange resin permits a sustained drug delivery system.
4. Microencapsulation or agglomerations are the methods to modify and control the release of active ingredient.

A. Composition of MCG's

Chewing gum is a mixture of natural or synthetic gums and resins, sweetened with sugar, corn syrup, artificial sweeteners and may also contain coloring agents and flavor. The basic raw material for all CG is natural gum Chicle, obtained from the sapodilla tree. Chicle is very expensive and difficult to procure therefore other natural gum or synthetic materials like polyvinyl acetate and similar polymers can be used as gum base.
Typically Chewing Gum comprises two parts
1. Water insoluble chewable gum base portion.
2. Water-soluble bulk portion.

1. **Water insoluble gum base :** They generally comprise of Elastomer, Resins, Fats and Oils, and Inorganic fillers.

a) Elstomers: Elastomer provides elasticity and controls gummy texture.
Natural elastomer: Natural rubbers like Latex or Natural gums such as Jelutong, Lechi Caspi, Chicle.

b) Plasticizers: These are used to regulate cohesiveness of product. These are again divided into c) **Natural and Synthetic**. Natural Plasticizers include Natural rosin esters like Glycerol Esters or Partially hydrogenated Rosin, Glycerol Esters of Polymerized Esters, Glycerol Esters of Partially dimerized Rosin & Pentaerythritol Esters of Rosin. Synthetic Plasticizers include Terpene Resins derived from α pinene and/or d-limonene.

d) **Fillers or Texturizers**: Provide texture, improve chewability, and provide reasonable size of the gum lump with low dose drug. Commonly used fillers are Magnesium and Calcium Carbonate, Ground Limestone, Magnesium and Aluminum Silicate, Clay, Alumina, Talc, Titanium Oxide & Mono/ di/ tri Calcium Phosphate.

2. **Water soluble portions:** These contain Bulk Sweeteners, High intensity Sweeteners, Flavoring agents, Softners, Emulsifiers, Colors & Antioxidants:

a) Softners and Emulsifiers: These are added to the chewing gum in order to optimize the chewability and mouth feel of the gum. Softners include Glycerin, Lecithin, Tallow, Hydrogenated Tallow, Mono/ di/ tri-Glycerides, Fatty acids like Stearic acid, Palmitic acid, Oleic acid and Linoleic acid.

b) Colorants and Whiteners: They may include FD & C type dyes and lakes, fruit and vegetable extracts, Titanium Dioxide.

c) Sweeteners: These are of two types, Aqueous and Bulk.

Aqueous Sweeteners can be used as softners to blend the ingredients and retain moisture. These include Sorbitol, hydrogenated Starch hydrolysates and Corn Syrups. Corn syrup keeps gum fresh and flexible.
Bulk Sweeteners include Sugar and Sugarless components. Sugar Components include Saccharides like Sucrose, Dextrose, Maltose, Dextrin, Fructose, Galactose, and Corn Syrup.
Sugarless Components include sugar alcohols such as Sorbitol, Manitol, Xylitol, hydrogenated Starch hydrolysate. High intensity artificial Sweeteners can also be included to provide longer lasting sweetness and flavor perception e.g. Sucralose, Aspartame, salt of Acesulfame

d) Bulking agents: These are used if low calorie gum is desired. Examples of low caloric bulking agents include Polydextrose, Oligofructose, Inulin, Fructo-oligosaccharides, Guar gum hydrolysate, Indigestible Dextrin.

e) Flavoring Agents: A variety of flavoring agents are used to improve flavor in chewing gum includes essential oils, such as Citrus oil, fruit essences, Peppermint oil, Spearmint oil, Mint oil, Clove oil & Oil of Wintergreen. Artificial flavoring agents can also be used.

f) Active Component: In medicated chewing gum active pharmacological agent may be present in core or coat or in both. The proportion of which may vary from 0.5-30% of final gum weight. A small, unionized, lipophilic and enzymatically stable active agent is likely to be absorbed more readily. Saliva soluble ingredient will be completely released within 10- 15 minutes of chewing whereas lipid soluble ingredient will dissolve in the gum base and thereafter be slowly and completely absorbed. (Heema N 2011)

B. Methods of preparation:

Methods employed for the manufacturing of Medicated Chewing Gums can be broadly classified into three main classes namely.
1. Conventional/ traditional Method (Melting).
2. Freezing, grinding and tabletting Method.
3. Direct Compression Method.

Conventional/ traditional Method:

Components of gum base are softened or melted and placed in a kettle mixer to which sweeteners, syrups, active ingredients and other excipients are added at a definite time. The gum is then sent through a series of rollers that form into a thin, wide ribbon. During this process, a light coating of finely powdered sugar or sugar substitutes is added to keep the gum away from sticking and to enhance the flavor. In a carefully controlled room, the gum is cooled for up to 48 hours. This allows the gum to set properly. Finally the gum is cut to the desired size and cooled at a carefully controlled temperature and humidity.

The conventional method has number of limitations like elevated temperature used in melting, restricts the use of this method for thermo labile drugs. Controlling of accuracy and uniformity of drug dose becomes difficult due to melting and mixing of highly viscous gum mass makes. Such a chewing gum composition is difficult to form into chewing gum tablets because of their moisture content (2-8%). If attempted to grind and tablet such a composition would jam the grinding machine, stick to blades, screens adhere to punches and would be difficult to compress. Technology not so easily adaptable to incorporate the stringent manufacturing conditions required for production of pharmaceutical products.

Cooling, Grinding and Tabletting Method:

This method has been developed with an attempt to lower the moisture content and to avoid the problems mentioned in conventional method.

Cooling, Grinding

The CG composition (base) is cooled to a temperature at which the composition is sufficiently brittle and would remain brittle during the subsequent grinding step without adhesion to the grinding apparatus. The temperature required for cooling is determined in part by the composition of the CG and is easily determined empirically by observing the properties of the cooled chewing gum composition. Generally the temperature of the refrigerated mixture is around - 15°C or lower. Amongst the various coolants like liquid nitrogen, solid carbon dioxide is preferred as it can give temperatures as low as -78.5°C. The refrigerated composition is then crushed or ground to obtain minute fragments of finely ground pieces of the composition. Additional solid carbon dioxide and silica are added to the ground composition, and the composition is further ground in a second grinding step. This two step grinding process advantageously keeps the chewing gum composition at a very low temperature. The presence of solid carbon dioxide also serves to enhance the efficiency of the grinding process. After the composition is ground to a powder, the coolant can be removed by allowing the coolant to evaporate.

Tabletting

Once the coolant has been removed from the powder, the powder can be mixed with other ingredients such as binders, lubricants, coating agents, and sweeteners etc, all of which are compatible with the components of the chewing gum base in a suitable blender such as sigma mill or a high shear mixer. Alternatively a Fluidized Bed Reactor (FBR) can be used. The use of FBR is advantageous as it partially rebuilds the powder into granules, as well as coats the powder particles or granules with a coating agent thereby minimizing undesirable particle agglomeration. The granules so obtained can be mixed with antiadherents like talc. The mixture can be blended in a V type blender, screened & staged for compression. Compression can be carried out by any conventional process like punching.

Use of anti-caking agent:

An anti-caking agent such as precipitated silicon dioxide can be mixed with chewing gum composition and solid carbon dioxide prior to grinding. This helps to prevent agglomeration of the subsequently ground chewing gum particles.

Use of grinding agents:

To prevent the gum from sticking to the grinding apparatus, 2-8% by weight of grinding aid such as alkaline metal phosphate, an alkaline earth metal phosphate or maltodextrin can be incorporated.

Direct Compression Chewing Gum:

Pharmagum is a compatible gum system that has been developed by SPI pharma. Pharmagum is a mixture of polyols and of sugar with gum base. Pharmagum® S consists primarily of gum base and Sorbitol. Pharmagum® M contains gum base, Manitol and Isomalt. It is a free flowing powder, which is directly compressible. This gum is manufactured under cGMP conditions and complies with food chemicals. Direct compression chewing gum can be directly compressed on a traditional tabletting machine, thus enabling rapid and low cost development of a gum delivery system. (Basani G 2011)

C. Factors Affecting Release Of Active Ingredient:

1. Contact Time:

The local or systemic effect is dependent on time of contact of Medicated Chewing Gum in oral cavity. In clinical trial chewing time of 30 minutes is considered close to ordinary use. The average chewing rate is about 60 chews every minute.

2. Physicochemical properties of active ingredient:

The saliva soluble ingredients will be immediately released within few minutes whereas lipid soluble drugs are released first into the gum base and then

Released slowly. Release of water soluble drug (aqueous solubility greater than 1:10) is, in general, about 75% or more during 5 min. of chewing and 90% or more during 15 min. of chewing at rate of 60 chews per minute. Drugs with aqueous solubility between 1:10 and 1:300 demonstrate up to 60% release during 10 minutes of chewing and between 50 to 90% when the gum is chewed for 15 min. The release of the drug, which is only slightly water-soluble, can only be expected to be small (less than 5%) even if the gum is chewed for 30 min

3. Inter individual variability:

The chewing frequency and chewing intensity which affect the drug release from Medicated Chewing Gum may vary from person to person. In-vitro study prescribed by European Pharmacopoeia suggest 60 cycles per minute chewing rate for proper release of active ingredient.

4. Formulation factor:

Composition and amount of gum base affect rate of release of active ingredient. If lipophilic fraction of gum is increased, the release rate is decreased.19 The influence of gum base mass on drug release has been investigated using Salicylamide as model drug. When Salicylamide was incorporated into a chewing gum, which contained a relatively large percentage of gum bases, the release after 30 min. of chewing was significantly lower (25.6%) compared to a gum in which less gum base was present (52%). (Nilima T 2011)

Evaluation of Medicated Chewing Gum:

Determination of drug release from medicated chewing gum:

Apparatus I. Chewing Gum Apparatus, Compendial— Ph. Eur.

The chewing apparatus for medicated chewing gum was adopted by Ph. Eur. in 2000. The chewing apparatus comprises a chewing chamber, two horizontal pistons, and a third vertical piston (tongue). **Figure 1** The vertical piston operates alternatively with the two horizontal pistons and makes sure the gum stays in the right place between chews. If necessary, it is feasible to construct the machine so that at the end of the chew the horizontal pistons rotate around their own axes in opposite directions to each other to obtain maximum chewing. The working procedure of this chewing apparatus is described in Ph. Eur. Several studies have been carried out using the Ph. Eur. apparatus, and the results indicate the methodology is rugged and reproducible.

Apparatus II. Alternative Chewing Gum Apparatus, Noncompendial—Wennergren

One of the Noncompendial apparatus commercially available was designed by Wennergren. The schematic representation of the Wennergren chewing apparatus is shown in **Figure 2**. The chewing procedure consists of reciprocations of the lower surface in combination with a shearing (twisting) movement of the upper surface that provides mastication of the chewing gum and at the same time adequate agitation of the test medium. The upper jaw has a flat surface that is parallel to the central part of the lower surface. The small brim of the lower surface is angled upwards (45 degrees) so that the lower surface functions as a small bowl with a flat bottom. This bowl prevents the chewing gum from sliding during mastication. (Jayachandar G 2008)

Physical evaluation: All formulations are physically evaluated for following parameters, Appearance, Color, Stickiness, Hardness / Plasticity, Weight variation, Drug Content

Hardness / Plasticity: Monsanto type hardness tester is used for determination of hardness / plasticity of all MCG formulations.

Weight variation: Weight of the ten chewing gums is taken from one batch then average weight is calculated from that standard deviation is calculated.

In-vitro drug release: All the formulations are studied for in-vitro drug release and the cumulative % of drug release are calculated. This is based on

i) Change in twisting angle of upper mastication jaw from (50 – 300).

ii) Change in distance b/w upper & lower masticating Jaw from (1-2mm).

iii) Change in chewing frequency of lower masticating Jaw from (20 strokes / minute to 120 strokes / minute).

iv) Change in temperature from (300C – 400C).

The chewing gum is inserted between the pistons on to the lower chewing surface. The chewing procedure consists of up and down stroke of lower masticating surface combined with twisting movement of upper masticating surface, thereby masticating the chewing gum and consequently agitating the test medium. At predominant time interval aliquot of the artificial saliva, is removed and assayed for drug content by UV spectrophotometric analysis. The release medium Is replaced with fresh artificial saliva after each sample was taken. (Farhad M 2011)

Stickiness: The MCG placed on the plain surface, mass of 250 gm Teflon hammer collide on it for period of ten minute. The frequency of hammering was about 30 / minute. After 10 minutes, sticking of mass to the hammered surface is observed and reported.

According to European Pharmacopoeia:-

Test for Uniformity of Content:

Unless otherwise prescribed or justified and authorized medicated chewing gum with content of 2 mg or less than 2 percent of the total mass of gum comply with test.

Uniformity of mass:

Uncoated medicated chewing gum and unless otherwise justified and authorized coated medicated chewing gum comply with the test for uniformity of mass of single- dose preparations.

Drug release from medicated chewing gum:

The drug release from medicated chewing gum as per the specification given in European Pharmacopoeia and is determined by applying a mechanical kneading procedure to a piece of gum placed in a small chewing chamber containing a known volume of buffer solution. (P 2011) (Shivanand P 2009)

***In Vitro* Drug Release Testing**

Standard dissolution apparatus is not suitable for monitoring release of drug from chewing gums as the action of chewing is essential, by providing a renewable surface for drug release after chew action. A gum formulation behaves differently and mastication is needed for the release of the drug compound. During the mastication process, the medication contained within the gum product should be released into the saliva and is either absorbed through the oral mucosa or swallowed and absorbed through the gastrointestinal tract. The chewed gum can then be removed and analyzed for the residual drug substance while pharmacokinetics can be determined from blood samples. A number of devices to mimic the chewing action have been reported. In 2000, the European Pharmacopoeia published a monograph describing a suitable apparatus for studying the in vitro release of drug substances from chewing gums.

There are two apparatus for studying release from chewing gum –

1) Masticator M 36

2) Instrument for In-vitro dissolution of medicated chewing gums 6-cell chewing apparatus.

Masticator M 36

Christrup and Moller's experiences prompted them to design a new chewing gum machine. This machine consists of a temperature-controlled reservoir for dissolution medium and two pistons. During one chewing cycle, the piston one on each side move towards each other, pressing the chewing gum between them and then make a twisting movement before returning to starting point. At specified time intervals, a sample is removed from the reservoir and quantity of drug released is determined. The cycle rate (chewing rate) is usually set at 60 per minute, and 20 ml of dissolution media equilibrated to 37° are typical volumes and temperatures of dissolution media.

Instrument for In-vitro dissolution of medicated chewing gums 6-cell chewing apparatus:

The apparatus designed by Christrup and Moller has been developed further which allows six pieces of chewing gum to be evaluated at the same time. In addition to the two horizontal pistons, the chewing chambers are supplied with vertical piston working staggered to the horizontal piston. This ensures that the gum is always positioned in the right place during the mastication process. One cycle of the piston consist of one upward and one downward stroke, with cycle frequencies of 7.5-30 every minute. Simultaneously, the plunger consistently rotates during the experiments at 10-40 rpm and a cycle frequency of 30 cycles per minute were found to give release rates similar to those observed *in vivo*. The apparatus of Christrup and Moller has been used in many studies with different formulations of chewing gums containing drug of wide range of aqueous solubility. In general the results have shown a high correlation between in vitro and in vivo release data. Moreover, the fact that six pieces of chewing gum can be examined at the same time is advantageous as it provides results at a faster rate and can be expected to be more reproducible than the results obtained with the original apparatus developed by Christrup and Moller. The 6-cell chewing apparatus is the world's only commercially available instrument for in-vitro dissolution of chewing gums. (Nilima T 2011) (Catharina K 2011)

Applications:

1. Dental caries:

1. Sugar free chewing gum is known to be beneficial to dental health. Prevention and cure of oral disease are targets for chewing gum formulations.
2. It can control the release rate of active substances providing a prolonged local effect.

3. It also re-elevates plaque pH which lowers intensity and frequency of dental caries.
4. Fluoride containing gums have been useful in preventing dental caries in children and in adults with xerostomia.
5. Chlorhexidine chewing gum can be used to treat gingivitis, periodontitis, oral and pharyngeal infections.
6. It can also be used for inhibition of plaque growth.

2. Systemic therapy:

1. Pain- chewing gum can be used in treatment of minor pains, headache and muscular aches.
2. Obesity- Active substances like chromium and caffeine are proved to be efficient in treating obesity. Chromium is claimed to reduce craving for food due to an improved blood-glucose balance. Caffeine and stimulate lipolysis and have a thermogenic effect (increased energy expenditure) and reduce feeling of hunger.
3. Other indications- Xerostomia, Allergy, Motion sickness, Acidity, Cold and Cough, Diabetes, Anxiety, etc
4. Clinical trials involving patients with oral candidiasis have shown that Miconazole chewing gum is at least as efficient as miconazole oral gel in the treatment of fungal infections in the mouth. Furthermore, patients preferred chewing gum to oral gel due to convenience and fewer side effects.
5. Several studies have been performed regarding the possibility of obtaining a constant, prolonged level of active substance locally for the treatment of oral fungal infections (S 2010) (P 2011) (Hitesh J 2010) (Saul S 2009)

6. Smoking cessation- Chewing gum formulation containing nicotine and lobeline have been clinically tested as aids to smoking cessation. A clinically limiting feature of commercially available nicotine gum is its slow rate of nicotine absorption leading to its slow onset of therapeutic effects. While nicotine gum is labeled for use at regular intervals, to reduce overall levels of craving and withdrawal.
7. Chlorhexidine chewing gum offers numerous flexibility in its formulation as it gives less staining of the teeth and is distributed evenly in the oral cavity.
8. The bitter taste of Chlorhexidine can be masked quite well in a chewing gum formulation.

Future Trends:

Chewing gum is no longer seen simply as confectionary. It not only offers clinical benefits but also is an attractive, discrete and efficient drug delivery system. A few decades ago, the only treatment for some diseases was surgical procedure but now more and more diseases can be treated with Novel Drug Delivery Systems. Generally, it takes time for a new drug delivery system to establish itself in the market and gain acceptance by patients, however chewing gum is believed to manifest its position as a convenient and advantageous drug delivery system as it meets the high quality standards of pharmaceutical industry and can be formulated to obtain different release profiles of active substances. The potential of MCG for buccal delivery, fast onset of action and the opportunity for product line extension makes it an attractive delivery form. Reformulation of an existing product is required for patent protection, additional patient benefits and conservation of revenues. Dental health chewing gum is here to stay, as is medicated gum for smoking cessation and travel sickness. More and more drugs are being formulated into chewing gum in preference to other delivery systems to deliver drugs locally to the oral cavity. The reason is simple that the chewing gum delivery system is convenient, easy to administer anywhere, anytime and its pleasant taste increases the product acceptability and patient compliance.

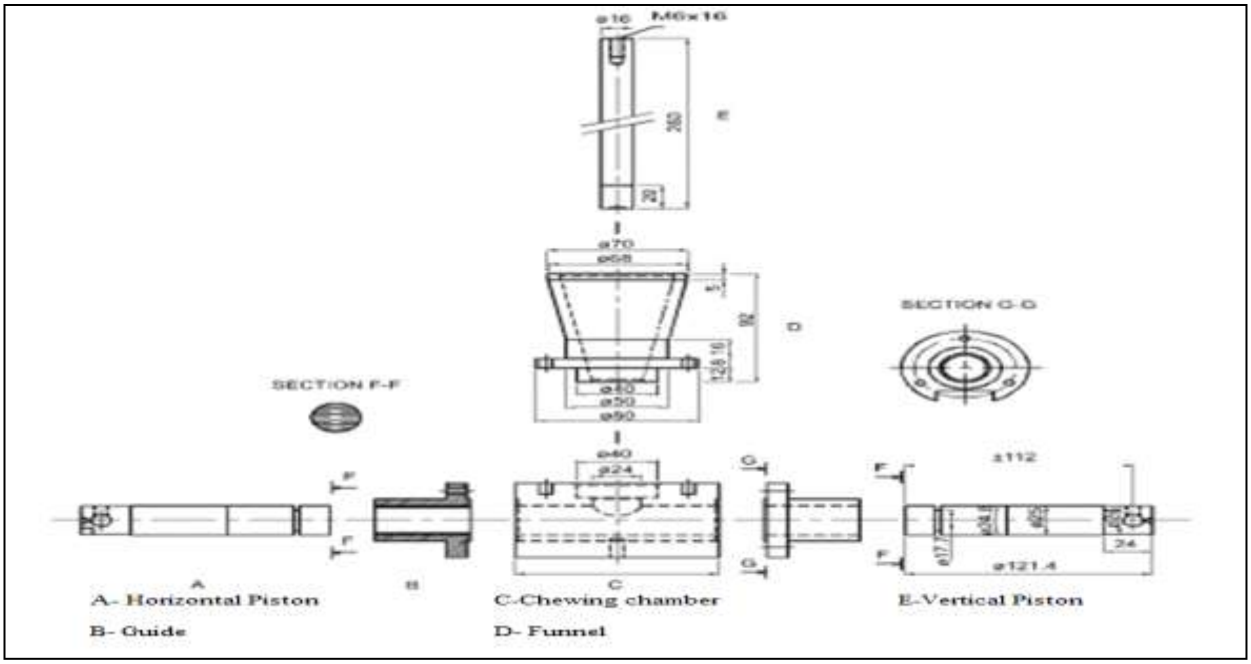

Figure1:- Apparatus for the determination of drug release from medicated chewing gum.

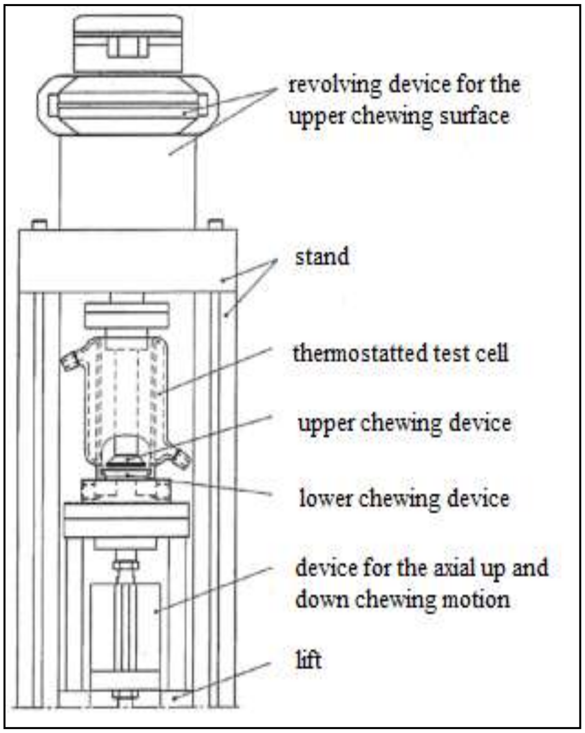
revolving device for the upper chewing surface
stand
thermostatted test cell
upper chewing device
lower chewing device
device for the axial up and down chewing motion
lift

Figure 2. Single-module chewing apparatus from Wennergren

Marketed Preparations:

Product	Drug
Nicorette	Nicotine
Nicotinell	Nicotine
NiQuitin CQ	Nicotine
Fluorette	Fluoride
Vitaflo CHX	Chlorhexidine
Advanced+	Chlorhexidine
HEXIT	Chlorhexidine
Stay Alert	Caffeine
Travvell	Dimenhydrinate

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ORAL THIN FILMS

Bhuruk Manisha, Satam Madhavi, Hastak Vishakha

Introduction:

Definition: (htt)

Oral thin films: Oral thin film drug delivery systems are solid dosage forms, which dissolve in a short period of time when placed in the mouth without drinking water or chewing. These are also called as Fast dissolving Oral films ,Buccal films/ Oral strips

Physicochemical properties of the oral mucosa: (Javier O. Morales 2011), (M.D. Nehal Siddiqui 2011), (Nagar.P 2011)

The oral mucosa presents differently depending on the region of the oral cavity being considered. The masticatory mucosa covers those areas that are involved in mechanical processes, such as mastication or speech, and includes the gingival and hard palate. This masticatory region is stratified and has a keratinized layer on its surface, similar to the structure found at the epidermis, and covers about 25% of the oral cavity. The specialized mucosa covers about 15%, corresponding to the dorsum of the tongue, and is a stratified tissue with keratinized as well as non-keratinized domains. Finally, the lining mucosa covers the remaining 60% of the oral cavity, consisting of the inner cheeks, floor of the mouth, and underside of the tongue. This lining epithelium is stratified and non-keratinized on its surface. The buccal mucosa covers the inner cheeks and is classified as part of the lining mucosa, having approximately 40–50 cell layers resulting in an epithelium500–600 lm thick. The epithelium is attached to underlying structures by a connective tissue or lamina propia,separated by a basal lamina. These lining mucosa and the lamina propia regions provide mostly mechanical support and no major barrier for penetration of actives.

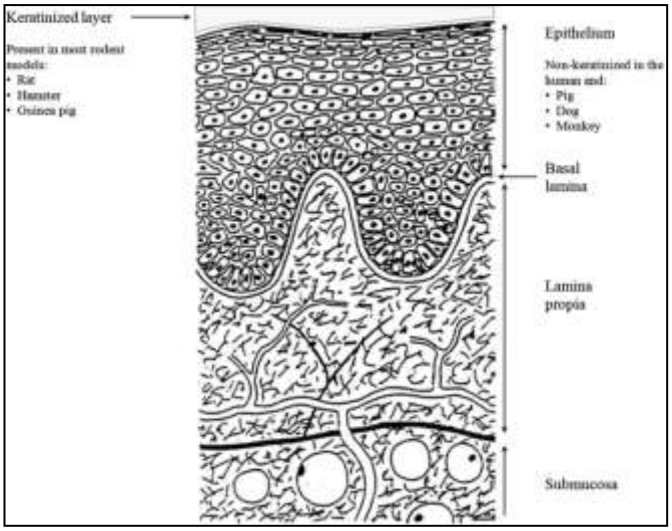


Figure-1: Diagram of a cross section of the buccal mucosa:

The keratinized layer is only present in most rodent models while the human has a non-keratinized buccal mucosa. The connective tissue also contains the blood vessels that drain into the lingual, facial, and retro mandibular veins, which then open into the internal jugular vein . This is one of the main advantages of buccal over oral delivery: absorption through the buccal epithelium avoids the gastrointestinal tract conditions, such as gastric pH, enzyme content, and the first pass effect due to direct absorption into the portal vein. Once a given drug molecule reaches the connective tissue, it may be readily distributed, thus the permeation barrier is across the whole thickness of the stratified epithelium .The existence of membrane-coating granules in the epidermis has been well characterized and it is known to be the precursor of the keratin layer or stratum corneum . Even though the existence of approximately 2 micrometer in diameter cytoplasmic membrane-coating granules in the buccal epithelium has been proven, Less is known in terms of their function; however, the permeation barrier is believed to be related to the presence of membrane coating granules in the buccal mucosa. More recently, it has been reported that some of these granules also contain lipid lamellae domains organized to some extent. This fact contrasts with the content of the membrane-coating granules in the epidermis, which contains very organized, electron-dense lipid lamellae. Therefore, the intercellular space of the stratified non-keratinized buccal mucosa is filled with a combination of amorphous material presenting some domains where short stack of lipid lamellae can be observed. This important difference in the intercellular space composition is responsible for the difference in permeability between the buccal and keratinized mucosa for exogenous compounds. Although the buccal mucosa is more permeable than keratinized epithelium, the existence of a permeability barrier has been described. It was demonstrated that this barrier is located in the upper one-third to one-quarter of the epithelium layer using horseradish peroxidase, and by following its permeation through the epithelium. After topical application, the horseradish peroxidise only permeated through the first 1–3 cell layers. However, when injected sub epithelially, it was found to permeate through as deep as the connective tissue and up as far as the membrane coating granules zone was. This suggested that the permeability barrier is located in the upper region of the epithelium and is correlated with the rich lipid content of this zone. As well as the keratinized epithelium, the intercellular space of the buccal mucosa is rich in lipids, but it is the difference in composition and the absence of the keratin layer that accounts for its permeation characteristics. The lipid composition in the buccal epithelium has a higher content of phospholipids, cholesterol esters, and glycosylceramides, while the content of ceramides is minimal, compared to the skin and keratinized regions of the oral cavity. This composition results in a higher concentration of polar lipids in the intercellular space. Therefore, it is not only due to the highly organized lipid lamellae found in the keratinized epithelia, but also the nature of the lipid content that accounts for the increased permeation of the buccal mucosa compared to the skin and other keratinized epithelia. Due to the polar nature of the lipids in the intercellular space, two different domains can be differentiated in the buccal epithelium: the lipophilic domain, corresponding to the cell membranes of the stratified epithelium, and the hydrophilic domain, corresponding to the extruded content from the membrane-coating granules, into the intercellular space. These two domains have led to postulate the existence of different routes of transport through the buccal epithelium, namely the paracellular and the transcellular route. The lipophilic nature of the cell membranes favors the pass of molecules with high log P values across the cells. Similar to the absorption mechanism in the small intestine, it is believed that lipophilic molecules are carried through the cytoplasm . However, there still is a lack of evidence supporting this assumption. The polar nature of the intercellular space favors the penetration of more hydrophilic molecules across a more tortuous and longer path . It has been demonstrated that some hydrophilic molecules are subject to carrier-mediated transport through the buccal mucosa. Most of the descriptions of molecules permeating through the buccal epithelium, in the literature, are related to the paracellular route of absorption. In an early study, it was found that tritiated water permeated through the paracelluar route . Using light microscopy autoradiography, it has been determined that water, ethanol, cholesterol, and thyrotropin release hormone penetrate through the paracellular route as well. More recently, it was demonstrated using confocal laser scanning microscopy that dextrans with 4 and 10 kDa average molecular weight and labeled with fluorescein isothiocyanate permeated through the paracellular route.

Even though there is no evidence that supports the idea of molecules permeating through the transcellular route, it is important to assess and understand the permeation route in order to determine strategies to enhance the absorption of actives when formulating buccal films.

Special features of mouth dissolving films: (Arun Arya Jan-Mar 2010)

- Thin elegant film
- Available in various size and shapes
- Unobstructive
- Excellent mucoadhesion
- Fast disintegration
- Rapid release

Advantages (Arun Arya Jan-Mar 2010), (Agarwal Jyoti 2011)

- Convenient dosing
- No water needed
- No risk of chocking
- Taste masking
- Enhanced stability
- Improved patient compliance.

A large number of drugs can be formulated as mouth dissolving films. Innovative products may increase the therapeutic possibilities in the following indications3.

-Paediatrics (antitussives, expectorants, antiasthamatics)
- Geriatrics (antiepileptic, expectorants)
- Gastrointestinal diseases
- Nausea (e.g. due to cytostatic therapy)
- Pain (e.g. migraine)
- CNS (e.g. antiparkinsonism therapy)

Advantages of oral dissolving film over oral disintegrating tablet:
Table 1:

Oral dissolving film	**Oral disintegrating tablets**
It is film	It is tablet
Greater dissolution due to greater surface area	Lesser dissolution due to less surface area
More durable	Less durable
Low patient compliance	Less patient compliance
No fear of chocking	Fear of chocking
They are not fragile,friable	They are fragile,friable

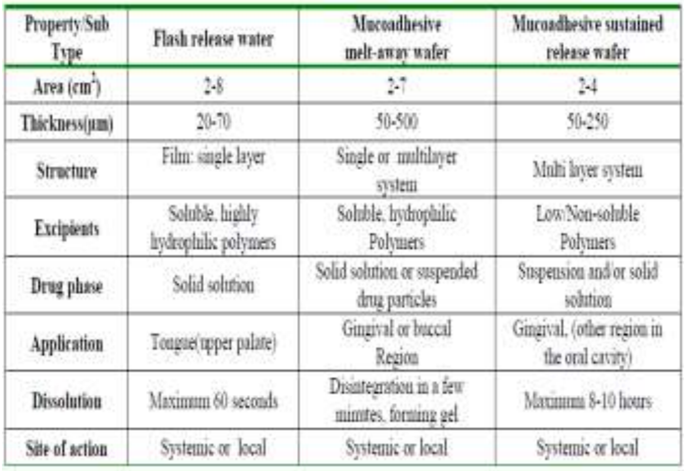
Classification of Oral Film: (Alpesh R. Patel1 April-June 2010), (Agarwal Jyoti 2011)

There are three different subtypes:

(1) Flash release
(2) Mucoadhesive melt-away wafer
(3) Mucoadhesive sustained-release wafers

These three types of oral films are differentiated from each other in following table

Table 2: Types of wafers and their properties



Property/Sub Type	Flash release water	Mucoadhesive melt-away wafer	Mucoadhesive sustained release wafer
Area (cm^2)	2-8	2-7	2-4
Thickness(μm)	20-70	50-500	50-250
Structure	Film: single layer	Single or multilayer system	Multi layer system
Excipients	Soluble, highly hydrophilic polymers	Soluble, hydrophilic Polymers	Low/Non-soluble Polymers
Drug phase	Solid solution	Solid solution or suspended drug particles	Suspension and/or solid solution
Application	Tongue(upper palate)	Gingival or buccal Region	Gingival, (other region in the oral cavity)
Dissolution	Maximum 60 seconds	Disintegration in a few minutes, forming gel	Maximum 8-10 hours
Site of action	Systemic or local	Systemic or local	Systemic or local

Composition of the oral thin film: (Arun Arya Jan-Mar 2010), (BASANI GAVASKAR 2010), (NARASIMHA RAO R JULY-SEPT2011)

Mouth dissolving film is a thin film with an area of 5-20 cm2 containing an active ingredient. The immediate dissolution, in water or saliva respectively, is reached through a special matrix from water-soluble polymers. Drugs can be incorporated up to a single dose of 15mg. formulation considerations (plasticizers etc.) have been reported as important factors affecting mechanical properties of the films, such as shifting shifting the glass transition temperature to lower temperature2. A typical composition contains the following Drug 1-25% Water soluble polymer 40-50% Plasticizers 0-20% Fillers, colours, flavours etc. 0-40%.

1) Drugs:

Several classes of drugs can be formulated as mouth dissolving films including antiulcer (e.g. omeprazole), antiasthamatics (salbutamol sulphate), antitussives, expectorants, antihistaminics, NSAID’S (e.g.paracetamol, meloxicam, valdecoxib).

2) Water soluble polymers:

Water-soluble polymers are used as film formers. The use of film forming polymers in dissolvable films has attracted considerable attention in medical and nutraceutical application. The water-soluble polymers achieve rapid disintegration, good mouthfeel and mechanical properties to the films. The disintegration rate of the polymers is decreased by increasing the molecular weight of polymer film bases. Some of the water soluble polymers used as film former are HPMC E-3 and K-3, Methyl cellulose A-3, A-6 and A-15, Pullulan, carboxmethylcellulose cekol 30, Polyvinylpyrollidone PVP K-90, Pectin, Gelatin, Sodium Alginate, Hdroxypropylcellulose,Polyvinylalcohol,Maltodextri nsandEUDRAGITRD.Polymerized rosin is a novel film forming polymer.

3) Plasticizers:

Formulation considerations (plasticizer, etc.) havebeen reported as important factors affecting mechanical properties of films. The mechanical properties such as tensile strength and elongation to the films have also been improved by the addition of plasticizers. Variation in their concentration may affect these properties. The commonly used plasticizers are glycerol, di-butylpthallate, and polyethylene glycols etc.

4) Surfactants:

Surfactants are used as solubilising or wetting or dispersing agent so that the film is getting dissolved within seconds and release active agent immediately. Some of the commonly used are sodium lauryl sulfate, benzalkonium chloride, bezthonium chloride, tweens etc. One of the most important surfactant is polaxamer 407 that is used as solubilizing, wetting and dispersing agent.

5) Flavour:

Any flavor can be added, such as intense mints, sour fruit flavors or sweet confectionery flavors.

6) Colour:

A full range of colours is available, including FD&C colours, EU Colours, Natural Colours and custom Pantone-matched colours.

7) Saliva stimulating agents:

Saliva stimulating agents Increases the saliva production rate, aids in faster disintegration of OTF Conc.- 2 -6 % w/w Examples – citric acid , malic acid, lactic acid, ascorbic acid, tartaric acid Flavoring agents: may be selected from syn. Flavor oils, oleoresins, from plant parts. Amount depends on the flavor type and strength Important flavors: peppermint, cinnamon, nutmeg, vanilla, cocoa, coffee, chocolate, citrus, apple, cherry, raspberry, pineapple.

Manufacturing Methods: (Arun Arya Jan-Mar 2010), (S. Malke 2010),

One or combination of the following process can be used to manufacture the mouth dissolving films.

i) Solvent casting
ii) Semisolid casting
iii) Hot melt extrusion
iv) Solid dispersion extrusion
v) Rolling

1) Solvent casting method:

In solvent casting method water soluble polymers are dissolved in water and the drug along with other Excipients is dissolved in suitable solvent then both the solutions are mixed and stirred and finally casted in to the Petri plate and dried.

Advantages:

- Better uniformity of thickness and better clarity than extrusion.
- Film has fine gloss and freedom from defects such as die lines.
- Film has more flexibility and better physical properties. The preferred finished film thickness is typically 12-100 μm, although various thicknesses are possible to meet API loading and dissolution needs.

Disadvantages:

1. The polymer must be soluble in a volatile solvent or water.
2. A stable solution with a reasonable minimum solid content and viscosity should be formed.
3. Formation of a homogeneous film and release from the casting support must be possible.
4. Multiple casting techniques may be selected on the basis of the fluid rheology, desired applied mass, and required dosage uniformity.

2) Semisolid casting:

In semisolid casting method firstly a solution of watersoluble film forming polymer is prepared. The 2resulting solution is added to a solution of acid Insoluble polymer (e.g. cellulose acetate phthalate, cellulose acetate butyrate), which was prepared in ammonium or sodium hydroxide. Then appropriate amount of plasticizer is added so that a gel mass is obtained. Finally the gel mass is casted in to the films or ribbons using heat controlled drums. The thickness of the film is about 0.015-0.05 inches. The ratio of the acid insoluble polymer to film forming polymer should be 1:4. Water soluble hydrocolloids dissolved in water to form homogenous viscous solution. Other ingredients including active agents dissolved in small portion of aqueous solvent using high shear processor. Both mixtures are mixed to form homogenous viscous solution and Degassed under vacuum. Bubble free solution is coated on non-treated casting film Coated film is sent to aeration drying oven .Film is cutted in to desired shape and size.

3) Hot melt extrusion:

In hot melt extrusion method firstly the drug is mixed with carriers in solid form. Then the extruder having heaters melts the mixture. Finally the melt is shaped in to films by the dies.

4) Solid dispersion extrusion:

In this method immiscible components are extrude with drug and then solid dispersions are prepared. Finally the solid dispersions are shaped in to films by means of dies.

Advantages:

- No need to use solvent or water.
- Fewer processing steps.
- Compressibility properties of the API may not be of importance.
- Good dispersion mechanism for poorly Soluble drugs.
- more uniform dispersion of the fine Particles because of intense mixing and Agitation.
- less energy compared with high shear Methods.

Disadvantages:

- Thermal process so drug/polymer stability problem
- Flow properties of the polymer are essential to processing
- Limited number of available polymers

5) Rolling Method:

In rolling method a solution or suspension containing drug is rolled on a carrier. The solvent is mainly water and mixture of water and alcohol. The film is dried on the rollers and cutted in to desired shapes and sizes. Other ingredients including active agents dissolved in small portion of aqueous solvent using high shear processor. Water soluble hydrocolloids dissolved in water to form homogenous viscous solution.

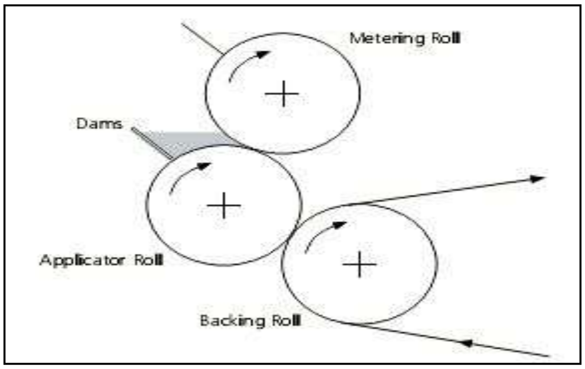


Figure 2: Three roll coating unit

Evaluating parameters:

1) Mechanical properties: (Arun Arya Jan-Mar 2010), (Amin Jan-Mar, 2011), (BASANI GAVASKAR 2010), (Alpesh R. Patel1 April-June 2010), (Sandeep Saini 2011), (Kulkarni A. S. 2010)

Mechanical properties of films are evaluated Instron using a TA.XT2 texture analyzer equipment equipped with a 5kg load cell. Films are held between two clamps positioned between 3cm. During measurement the strips were pulled at rate of 2mm/sec. The force and elongation were measured when film breaks. Three mechanical properties namely tensile strength, elastic modulus and % elongation are calculated.

a) Tensile strength:

Tensile strength is calculated by formula = force at break/ initial cross sectional area of film in mm^2.

b) Elastic modulus:

Elastic modulus is calculated by formula Elastic modulus = force at corresponding strain /Cross sectional area (mm2)*1/ Corresponding strain.

c) % Elongation:

It is calculated as = Increase in length/ Original length *100

d) Folding endurance:

Folding endurance is determined by folding the films of uniform cross sectional area and thickness until it breaks.

e) Film thickness:

Film thickness was measured by using a MI 1000 lm. The accuracy of the instrument was 2.5 lm± 0.5%. A 10 _ 2.5 cm sample of the film was placed between the anvil and the presser foot of the micrometer, and its thickness was measured in ten different positions. The determination was performed in triplicate.

2) Morphology study: (Arun Arya Jan-Mar 2010)

The surface morphology of the film forming excipient pullulan, cetirizine hydrochloride and films was observed using Environment scanning electron microscope. The film sample was placed in the sample holder and the photomicrographs were taken at 65x and 350x magnification using tungsten filament as an electron source.

3) Swelling property: (Arun Arya Jan-Mar 2010)

Film swelling studies is conducted using simulated saliva solution. Each film sample is weighed and placed in a preweighed stainless steel wire mesh. The mesh containing film sample is submerged into 15ml medium in a plastic container. Increase in the weight of the film was determined at preset time interval until a constant weight was observed. The degree of swelling was calculated using parameters **wt-w0/wo, wt** is weight of film at time t, and **wo** is weight of film at time zero.

4) Contact angle:

Contact angle measurements are performed at room temperature with a goniometer. A drop of double distilled water was placed on the surface of the dry film. Images of the water droplet were recorded within 10 seconds of deposition by means of digital camera. Digital pictures were analyzed by imageJ 1.28v software for angle determination. A minimum of five Measurements, taken at different positions of the film, was carried out. The contact angle was measured on both sides of the drop and averaged.

5) Taste evaluation: (Amin Jan-Mar, 2011)

Taste acceptability was measured by a taste panel consisting of human volunteers(n=6) with 10 mg drug and subsequently film sample containing 10 mg drug held in mouth until disintegration, then spat out and the bitterness level was recorded. The volunteers were asked to gargle with distilled water between the drug and sample administration. Following scale was used for the indicating taste masking values:
+ = very bitter,
++ = moderate to bitter,
+++ = slightly bitter,
++++ = tasteless/taste masked

7) Dryness test/tack tests: (BASANI GAVASKAR 2010)

About eight stages of film drying process have been identified and they are set-to-touch, dust-free, tack-free (surface dry), Dry-totouch, dry-hard, dry-through (dry-to-handle), dry-to-recoat and dry print-free. Although these tests are primarily used for paint films most of the studies can be adapted intricately to evaluate pharmaceutical OS as well 13. The details of evaluation of these parameters can be checked elsewhere and are beyond the scope of this review. Tack is the tenacity with which the strip adheres to an accessory (a piece of paper) that has been pressed into contact with the strip. Instruments are also available for this study.

8) In vitro dissolution studies: (Arun Arya Jan-Mar 2010)

The in vitro dissolution study is carried out in simulated saliva solution pH 6.4 phosphate buffer using USP paddle apparatus at 37±0.5°C. samples are withdrawn at regular time interval interval and analyzed by UV-Visible spectrophotometer.

9) Stability test: (Hiroyoshi Shimoda a 2009)

A piece of film preparation was stored in an aluminum package at 25 _C with 50–60% humidity (normal condition) or at 40 _C with 75% humidity (accelerated condition) for 4–24 weeks, then the content of drug was determined. In addition, the film sample was subjected to the dissolution test.

10) Determination of dissolution rate by conductivity method:(Arun Arya Jan-Mar 2010)

In the past 5 years several personal care products formulated in quick release film form have entered the marketplace, of which fast-dissolve breath fresheners were first. The fast dissolve oral films completely dissolve in as little as 1 minute. The majority of oral films on the market today contain ionisable components. This work presents a method for high resolution monitoring of the dissolution of fast dissolving oral films by measuring conductivity of the dissolution medium.

Equipment required

- Variable speed stirrer motor: capable of 250 rpm.
- Analytical balance (medium weight): capable of weighing to the nearest 0.01g and having a range not less than 400g.
- Low-form beaker: 3×800 ml.
- Impeller: 2-inch diameter, 1 blade (stainless steel or metal).
- Conductivity probe: capable of measuring conductivity to 0.1 μ siemens (Hann 8033 conductivity meter).
- Laboratory equipment stand.
- Double-sided clear tape: 3/4 inch-wide.
- External stands to hold conductivity probe.
- Scissors
- Stopwatch with a second hand.

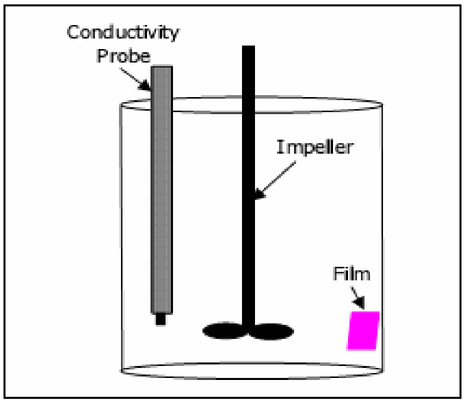


Figure-3: Diagram of equipment set-up. (Arun Arya Jan-Mar 2010)

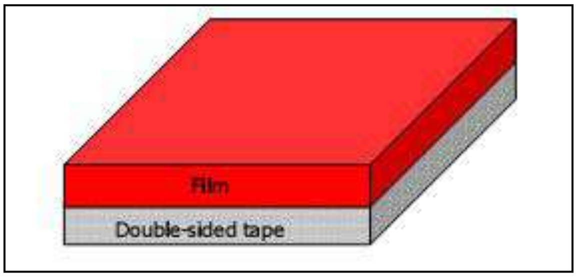


Figure 4: Diagram of prepared film. (Arun Arya Jan-Mar 2010)

Film Preparation:

One side of the film is adhered to the double sided tape, and the double sided tape is cut to the dimensions of the film (Figure. 3).

Test Procedure:

- Fill a clean beaker with 300 g (±0.05g) of the deionized water.
- Test the conductivity of the water to establish the background value.
- Adhere the film inside the dry, clean 800ml beaker so that the centre section is even with or slightly below the 100 ml line of the beaker. Arrange theConductivity probe and the impeller in the beaker.
- As quickly as possible pour the 300 ml of the water in the beaker containing the film, impeller and the conductivity probe. When the water completely covers the film, start the timer (approx 3sec). Then restart the impeller stirring at 250rpm.
- Take a data point at every 10 sec for the first minute. The take data as appropriate.

11) Disintegration time testing: (Alpesh R. Patel1 April-June 2010)

The disintegration time limit of 30 s or less for orally disintegrating tablets described in CDER guidance can be applied to fast dissolving oral strips. Although, no official guidance is available for oral fast disintegrating films/strips, this may be used as a qualitative guideline for quality control test or at development stage. Pharmacopoeial disintegrating test apparatus may be used for this study. Typical disintegration time for strips is 5-30 s .Furthermore, a disintegration measurement setup for fast-dissolving oral dosage forms, in this case ODTs, has been described but this setup cannot be transferred to oral wafers. For both methods only a small amount of medium is used, so natural conditions could be simulated. Due to the use of the Small amount of medium the dissolved drug substance could not be measured by spectral analysis.

(1) Slide frame method: one drop of distilled water was dropped by a Pipette onto the oral films. Therefore the films were clamped into slide frames and were placed planar on a Petri dish. The time until the film dissolved and caused a hole within the film was measured.

(2) Petri dish methods: 2 mL of distilled water was placed in a Petri dish and one film was added on the surface of the water and the time measured until the oral film was dissolved completely.

Packaging of Oral Film: (S. Malke 2010)
Expensive packaging, specific processing, and special care are required during manufacturing and storage to protect the dosage of other fast-dissolving dosage forms. Unlike these other quick-dispersing and/or dissolving oral delivery systems, the Quick-Dis™ system can be packaged using various options, such as single pouch, blister card with multiple units, and continuous roll dispenser, depending on the application and marketing objectives

Single pouch: Soluble Film Drug Delivery Pouch is a peelable pouch for "quick dissolve" soluble films with high barrier properties. The pouch is transparent for product display. Using a 2 structure combination allows for one side to be clear and the other to use a cost-effective foil lamination. The foil lamination has essentially zero transmission of both gas and moisture. The package provides a flexible thin film alternative for nutriceutical and pharmaceutical applications. The single dose pouch provides both product and dosage protection.

Blister card with multiple units: The blister container consists of two components: the blister, which is the formed cavity that holds the product, and the lid stock, which is the material that seals to the blister. The film selection should be based upon the degree of protection required. Generally the lid stock is made of aluminum foil. The material used to form the cavity is typically a plastic, which can be designed to protect the dosage form from moisture.

Polyvinyl Chloride:The most commonly used blister material is polyvinyl chloride (PVC). This material, which provides a nominal or zero barriers to moisture, is used when the product does not require effective moisture protection.

Barrier Films: Many drug preparations are extremely sensitive to moisture and therefore require high barrier films. Several materials may be used to provide moisture protection such as Polychlorotrifluoroethylene (PCTFE) film, Polypropylene.

Continuous roll dispenser: An automatic drug tape dispensing and metering device and a disposable cassette containing a roll of drug tape housed in a small reusable portable dispenser unit. The dispenser contains a measurement device for carefully measuring the length of tape as it is dispensed. A counter monitors the remaining doses of drug tape remaining within the dispenser. A timer device may be provided to alert the patient that it is time for the medicament to be dispensed. As the lid of the dispenser unit is opened, the measured length of drug tape is severed from the roll by a cutter blade incorporated into the lid. The dosage and administration of the medicament to be given a patient may be set by adjusting the tape length released for each single dose and selecting the time intervals between dosages. The invention comprises also ingestible tapes of medicament.

Applications of Oral Film Delivery System: (S. Malke 2010)

Taste masking: Oral film systems dissolve or disintegrate in patient's mouth, thus releasing the active ingredients which come in contact with the taste buds and hence, taste masking of the drugs becomes critical to patient compliance. An important aspect of thin film drug delivery technology is the masking of the often bitter and poor taste of drug formulations. One method of taste-masking is encapsulation, the coating of drug particles with a polymeric covering sufficient to mask the taste of the drug particle while maintaining the ability to release the drug for absorption.

Orally disintegrating films: Orally disintegrating thin film based on a water-soluble polymer. The film disintegrates rapidly within seconds in contact with water or saliva, releases the drug in the mouth and promotes gastrointestinal absorption.

Vaccination: Oral thin film is delivered in the form of vaccine which is stable at room temperature so that is quickly dissolve in mouth and in saliva. Rotavirus vaccine is prepared in United States by Johns Hopkins University in 2006. Rotavirus vaccine is a room temperature stable quick-dissolving oral thin film delivery system for vaccines that will make vaccinations almost as simple as freshening your breath. This delivery system exhibits many advantages not available in current products: improved patient compliance, improved bioavailability, reduction in the costs associated with storage, distribution, handling and administration.

Sustained release film: Sustained release oral film is applicable in hospital preparations and drug carriers by polymer Chitin and Chitosan derivatives are used as excipient and drug carriers in the pharmaceutical field. Their derivatization contributed to expansion of application and decrease toxicity. Chitosan is used as an excipient in oral dosage form. Films prepared using chitin or Chitosan have been developed as wound dressings, oral mucoadhesive and water-resisting adhesive by virtue of their release characteristics and adhesion.

Marketed Films: (Alpesh R. Patel1 April-June 2010), (Arun Arya Jan-Mar 2010)

S.No.	Product	Manufactured By
1.	Doneprezil rapid dissolving films, Ondansatron rapid dissolving films	Labtec Pharma
2.	Altoid cinnamon strips, Boots vitamin c strips, Cool shock peppermint strips, Benzocaine films, Caffeine films	Dow chemical company
3.	Listerine Pocket Paks Breath Freshening Strips	Pfizer's Warner-Lambert consumer healthcare division
4.	Klonopin Wafers	Solvay Pharmaceuticals
5.	Listerine Cool Mint Pocket Paks	Pfizer,Inc.
6.	Sudafed PE	Wolters Kluwer Health, Inc.
7.	Suppress®	InnoZen®, Inc.
8.	Triaminic	Novartis
9.	Theraflu	Novartis
10.	Orajel	Del
11.	Gas-X	Novartis
12.	Chloraseptic	Prestige
13.	Benadryl	Pfizer

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NAIL DRUG DELIVERY SYSTEM

Basrur Pooja, Suryavanshi Kiran, Katedeshmukh Ramesh

Introduction

Topical nail preparations like lacquers, varnish, enamel etc are generally used to enhance beauty of nails, imparting color and luster to nail. But in recent medicated lacquers are specially designed for the nail. These preparations are generally used in anti fungal diseases. Use of this system avoids oral toxicity of anti-fungal drugs.

Over the last decades the treatment of illness has been accomplished by administrating drugs to human body via various routes namely oral, sublingual, rectal, parental, topical, inhalation etc. Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin. The nail is horny structure. Nail plate is responsible for penetration of drug across it. As it is hard enough the penetration becomes difficult, only a fraction of topical drug penetrates across it. Hence the effective therapeutic concentration is not achieved. Infected nails appear slightly discolored, thickened, and dystrophic. The nail plate may appear abnormal as a result of decreased glow. Thus variety of diseases occurs. Onychomycosis (Tinea unguium) is a fungal infection of the nail bed or nail plate. Among superficial infections, Onychomycosis is the most difficult to manage and eradicate and it tends to recur. These diseases can be cured by achieving desired therapeutic concentration of drug by nail drug delivery system. In order to successfully deliver active pharmaceutical ingredients (APIs) across the nail it is necessary to consider the anatomy and physiology of barriers. To obtain the right amount of drug to the right place at the right time more effectively, newer drug delivery approaches can be utilized to maximize the effectiveness of the API – (Akomeah et al., 2004). Topical delivery of systemic therapeutics offers benefits but presents a greater technical challenge. The benefits include first pass avoidance, convenience and sustained release. Conventional nail lacquers have been used as cosmetics since a long time for beautification and protection of nails. Nail lacquer can be used as a drug delivery system for the drugs that exhibit poor oral bioavailability. Medicated nail lacquers are formulations that are used for transungual drug delivery system for maximal antifungal efficacy. The film on the nail surface acts as a drug depot that permits optimized and sustained diffusion across the nail and leads to continuous penetration of active principle to high tissue concentration required for the efficacy for the treatment of Onychomycosis.

Advantages

Preparation is easy compared to oral dosage forms like tablets etc. Drug interactions – drug interactions are absent. Adverse effects – systemic adverse effects are absent. The less common local rash related side effects like periungual erythema and erythema of the proximal nail fold gradually disappear after a few minutes and usually get less over time as your body adjusts to the new medication. Removal – systemic absorption is less and as it is a topical formulation it can be easily removed when needed. Possible improved adherence Suitable for patients who are unable to take systemic medication. It is preferred in elderly patients receiving multiple medications, to avoid drug-drug interactions. Multiple classes of antifungal medications have been utilized. Topical drug delivery is especially suitable for Onychomycosis (fungal infections of the nail plate and/or nail bed) and nail psoriasis, which affect 2 - 13 and 1 - 3% of the general population, respectively, and make up the bulk of nail disorders. Topical therapy would avoid the adverse events and drug interactions of systemic antifungal agents and the pain of injection when antipsoriatic agents are injected into affected nail folds. It cannot be easily removed by rubbing, washing etc. Nail lacquers can form depot. In addition, the effect is long lasting. A single application of lacquer provides protection for 1 week. Release and rate of diffusion can be optimized by selecting the components of lacquer formulation (solvent, polymer and plasticizer). However, successful topical therapy is extremely challenging due to the very low permeability of the nail plate. (S. Firoz 2011)

Major Challenges

- The nail plate is thicker which creates longer diffusional pathway for drug delivery. Stable disulphide bonds are responsible for the hardness of the nail, which restrict drug penetration. Potential penetration enhancers can be used to permeate formulations inside the nail barrier to deliver the active principle.
- It is essential to consider the physicochemical properties of the drug molecule (e.g. size, shape, charge log P etc.), the formulation characteristics (e.g. vehicle, pH drug concentration), possible interactions between the drug and keratin and possible penetration enhancer when designing topical formulations for nail drug absorption. (Kenneth et al., 1983)
- In oral antifungal therapy, liver function tests have to be performed regularly. Such therapies are therefore costly and are also hindered by poor patient compliance. Thus topical therapy remains the treatment of choice.

Factors which influence drug transport into and through the nail plate

a) Molecular size of diffusing molecule

Molecular size has an inverse relationship with penetration into the nail plate. The larger the molecular size, the harder it is for molecules to diffuse through the keratin network and lower the drug permeation.

b) Nature of vehicle

Water hydrates the nail plate which consequently swells. Considering the nail plate to be a hydrogel, swelling results in increased distance between the keratin fibers, larger pores through which permeating molecules can diffuse and hence, increased permeation of the molecules. Replacing water with a non-polar solvent, which does not hydrate the nail, is therefore expected to reduce drug permeation into the nail plate.

c) pH of vehicle and solute charge

The pH of aqueous formulations affects the ionization of weakly acidic/basic drugs, which in turn influences the drug's hydrophilicity/hydrophobicity, solubility in the drug formulation, solubility in the nail plate and its interactions with the keratin matrix. There have been conflicting reports in the literature on the influence of pH. Walters et al. (1985b), studied the permeation of the weakly basic drug, miconazole, through hydrated human nail plate. The permeability coefficient of the drug was found to be essentially the same at all pH studied i.e. there was no effect of pH and of drug charge on its permeability coefficient. In other studies, pH of the medium was found to have a distinct effect on drug permeation. The permeation of benzoic acid through the nail plate is different at different pH. The donor cells containing saturated solutions of the permeate is matched with pH of the receptor phase. It was found that as the pH of the medium was increased from 2.0 to 8.5, the permeability coefficient of benzoic acid decreased by 95.5% and the lag time increased.

d) Enhancement of drug permeation into nail

Applied drugs must permeate through the dense keratinized nail plate and reach the deeper layers of the nail plate, nail bed and the nail matrix. The nail plate has a low permeability and drug permeation has to be assisted. This can be done by physical and/or chemical means. Physically, removing part of the nail plate by filing reduces the barrier that drugs have to permeate through to reach the target sites. In clinical trial studies, the physical elimination of part of the nail plate prior to the application/reapplication of drug-containing formulations was essential for the success of topical treatment. The dorsal layer of the nail plate is the main barrier to drug diffusion into the nail plate. Filing the dorsal layer of nail clippings from healthy volunteers increased drug permeation. Filing the ventral layer also increased drug permeation, though to a lesser extent. Of course, in practice, one can only file the dorsal layer of nail plates. Two main ways of increasing ungual drug transport that have been investigated are:

(i) The use of agents such as urea and salicylic acid, which soften nail plates; and

(ii) The use of sulfhydryl compounds such as cysteine which cleave the disulphide linkages of nail proteins and destabilize the keratin structure. (Vikrant N 2011) (Vivek R 2012)

Structure of Nail

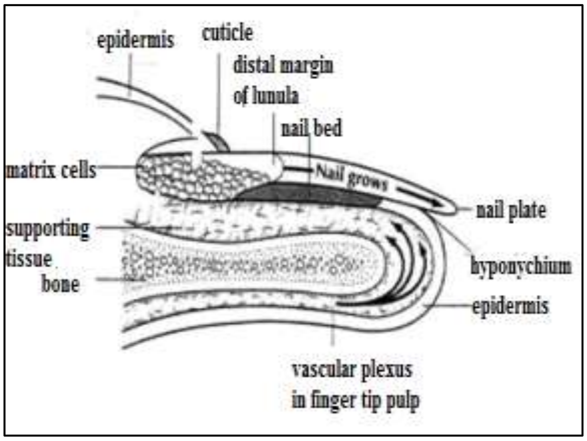


Figure-1 Semi-Diagrammatic Cross Section Of Finger Nail

The nails are composed of flat, horny scales which form protective covering for the distal of the finger & toes. Each nail consist of following parts -
1) A body, the attached uncovering of the nail
2) A free edge, the anterior unattached extension of the body
3) The nail root, the posterior or proximal part of the nail, which lies beneath a fold of the skin.

Most of the body of the nail is pink because it is sufficiently translucent to transmit the color from the underline vascular tissue. The proximal part of the nail is whitish & called the lunula because of it has pale moon shape due to the air mixing with the keratin matrix. It has no physiological function. The fold of skin, which extends around the proximal & lateral borders of the nail, constitutes the nail fold, & the skin, which lies beneath the nail, forms the nail bed. The furrow between the nail bed & nail fold is the nail groove. The nail itself is a hard horny & consists of several layers of clear, flat cells that contain shrunken & degenerated nuclei. The striated appearance observed in section cut perpendicular to the surface is produced by the arrangement of the cell in layer.

The bed consist of epithelium and dermis continuous with the epidermis & dermis if the skin of the nail folds. The epidermis of the nail folds usually has zones characteristic of palmar skin, although the stratum lucidum may thin or absent in some cases. The stratum corneum of the proximal nail fold turns into the nail groove, spreads over the upper surface of the root, and is continuous for a short distance on to the surface of the body of the nail as the eponychium.

The epithelium of the nail bed corresponds to Malpighian layer of the of skin & like the later, consists of polygonal prickle cell & stratum cylindricum resting upon a basement membrane. The epithelium of the posterior part the nail bed, the part that lies beneath the root & the proximal portion of the body corresponding to the lunula, is thicker than elsewhere & is called as the matrix because it functions for nail growth. Growth of nail takes place by a transformation of the more superficial cells of the matrix in to true nail cells. In the process the outer, harder layer is pushed forward over the Malpighian layer. The nails generally increase in length by about 0.5 mm / week. Fingernails grow more quickly than toenails & growth is quickly when the environmental temperature is high. Nail plate is almost completely formed by the 20th week of the fetal life .Under the distal free edges of nail, the epithelium of the nail bed become continuous with the malpighian layer of skin & the other layers characteristic of the skin thickened and is known as the hyponychium .

The dermis of the nail bed differs somewhat from that of ordinary skin. Its connective tissue fibers are arranged partly longitudinal to the long axis of the nail & partly in a vertical plane extending from the posterior to the nail. Dermal papillae are found beneath the proximal part of the nail root but disappear beneath the distal part of the root, to be replaced by longitudinal dermal ridges which, increase in height as they pass forward, continued to the distal end of the nail bed. Because of the dermal ridges longitudinally, the boundary between the epithelium and connective tissue appears smooth in longitudinal section and irregular and papilla like in cross section.

The human nail plate consists of three layers; the dorsal & intermediate layer derived from the matrix, & the ventral layer from nail bed. The intermediate layer is three - quarter of the whole nail thickness &

consists of the soft keratin.

The upper hard layer consists of hard keratin which constitutes 94 % by weight of nail. The upper layer of the nail mainly diffuses into & through the Nail plate. The ventral layer consists of soft hyponychial in which changes occurs. Thus, in the treatment of these nail diseases; an effective drug concentration in the ventral nail plate would be of great importance.

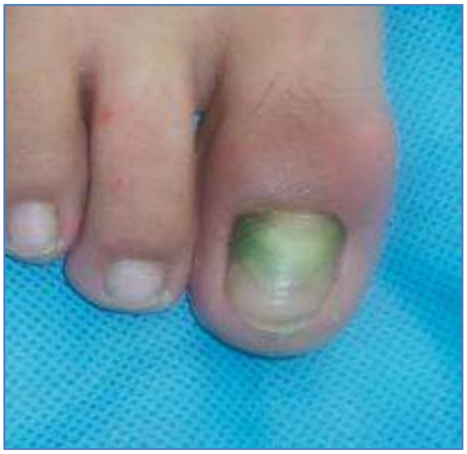
Nail Growth is greatest in childhood and decrease slowly with aging. Due to pressure from posterior nail fold the grows forward instead of upward. Nail growth is also affected by local disturbances in the nail fold or by abnormal keratinization of the nail plate. General or local factor may result in the development in the nail of thicken, ridging, pitting, discoloration, brittleness, splitting, and even separation of nail from its bed (onycholysis). A transverse groove may result from severe illness. The changes in color for a variety of reasons for instance white spots in the nail plate, which is seen 62% of normal people, is due to imperfect keratinization with retention of nuclear material. (Bothiraja C 2006)

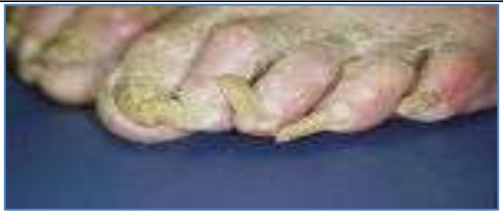
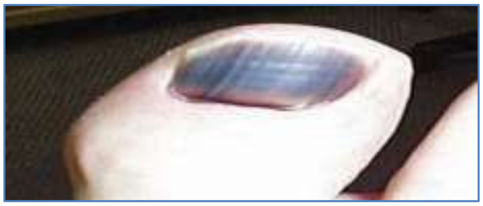
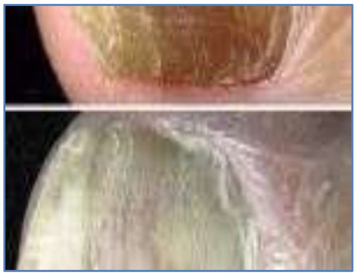
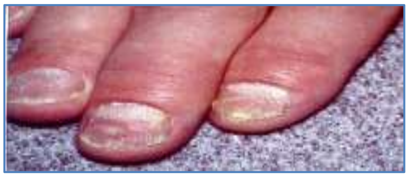
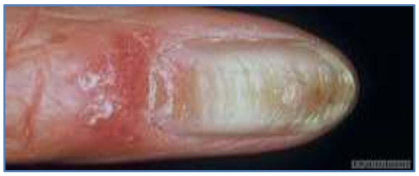
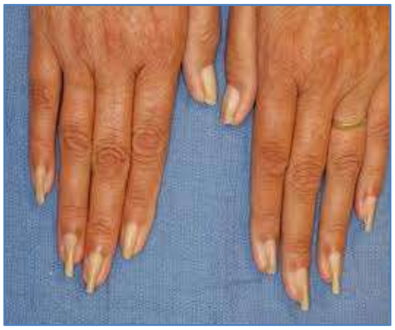
(Skin and Nail: Barrier Function, Structure, and Anatomy Considerations for Drug Delivery 2009)

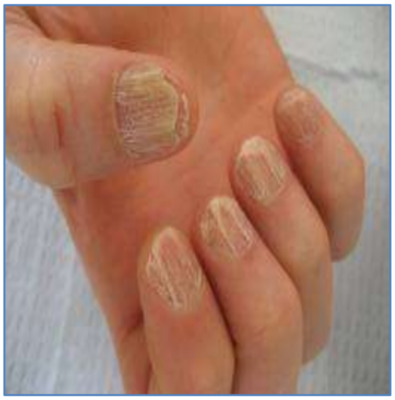
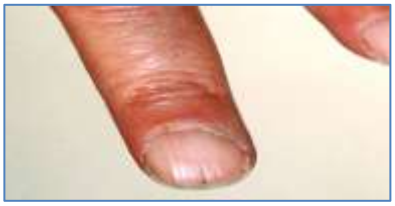
Physiochemical Characterization of the Human Nail:

The plate consists of laminated sheets of keratinized cells. Thin lipid seams separate the layer. The lipids form the original cell membrane coating granules during the plate formation. No evidence exists concerning fundamental permeation mechanisms & possible influence of chemical structure on transport across the nail plate. Nail plate permeability coefficients can be determined using standard diffusion cell techniques. The hydrated human nail plates behave like a hydrogel of high ionic strength to the polar & semi polar alcohols, declining permeability hydrophobic species. So the nail become less permeable to the n-alcohols as their hydrophobicity is increased. (Bothiraja C 2006)

Diseases of Nail (Wingfield.R 2009)

Disorders	Description of Disorder	Treatment available	Images of the disorder
Green-nail syndrome	Infection is caused by Pseudomonas. It is generally a harmless infection, usually of 1 or 2 nails, and is noteworthy for its striking blue-green color.	Treatment is most effective with soaks of 1% acetic acid solution or alcohol diluted 1:4 with water. Patients should soak their affected nails twice a day for 10 min and should avoid trauma and excess moisture. Frequent clipping of the nail increases the response to treatment.	

Onychogryphosis	It is a nail dystrophy in which the nail, most often on the big toe, becomes thickened and curved.	Treatment consists of trimming the deformed nails.	
Subungual hematoma and nail bed trauma	Subungual hematoma occurs when blood becomes trapped between the nail plate and nail bed, usually as a result of trauma. It causes significant pain and eventual separation of and temporary loss of the nail plate.	If the injury is acute, nail trephination (e.g., creating a hole in the nail plate using a cautery device, 18-gauge needle, or red-hot paperclip) can help relieve pain by draining accumulated blood.	
Onychomycosis	Onychomycosis is fungal infection of the nail plate, nail bed, or both Toenails are 10 times more commonly infected than fingernails.	Topical antifungal nail lacquer containing ciclopirox 8% or amorolfine 5% is rarely effective as primary treatment but can improve cure rate when used as an adjunct with oral drugs, like terbinafine or itraconazole	
Psoriasis	nails may have a number of changes, including irregular pits, oil spots	Corticosteroids, salicylic acid, calcipotriol or tazarotene help treat it. These may be given alone or in combination.	
Onychotillomania	In this disorder, patients pick at and self-mutilate their nails, which can lead to parallel transverse grooves and ridges	---	
Pincer nail deformity	Pincer nail deformity is a transverse over-curvature of the nail plate. It can occur in patients with psoriasis, SLE, Kawasaki disease, cancer, end-stage renal disease, and some genetic syndromes.	---	

Trachyonychia	Trachyonychia (rough, opaque nails) may occur with alopecia areata, lichen planus, atopic dermatitis, and psoriasis. It is most common among children.	---	
Splinter hemorrhages	These are linear hemorrhages lying parallel to the long axis of finger or toe nails.	---	

Drugs for Antifungal Therapy

The antifungal agents act on various targets. Drugs acting on the cell membrane include polyene antibiotics like Amphotericin B lipid formulations, Nystatin (topical) and azole antifungals like, Ketoconazole, Itraconazole, Fluconazole, Voriconazole, Miconazole and Clotrimazole. DNA synthesis is another target for antifungal therapy, and this therapy includes drugs like Pyrimidine analogues, e.g. Flucytosine. The antifungal drugs that act on cell wall are Echinocandins, Caspofungin acetate.

Enhancing Drug Delivery through Human

Nail Drug permeation into the nail plate is normally very low as the nail plate is a good barrier. Since the nail plate's permeability is so low, a drug candidate must be effective at low concentrations i.e. have a very high potency. The drug must be released from the lacquer film and penetrate into the nail plate before it can act. A small molecular size also helps; drug flux through the nail plate is found to be inversely proportional to molecular size.

Increasing the drug concentration in the lacquer increases drug flux in the nail. Increasing the frequency of lacquer application, from once to twice a week results in slightly increased mycological cure rate. The nature of the drug formulation is expected to influence drug partitioning from the vehicle into the nail plate.

Absorption through Nail:

In Vitro Nail (Ungual) Absorption

Dr Clive Roper of Charles River Preclinical Services has developed a model to monitor invitro ungual absorption. This *in vitro* model provides key information regarding formulation selection and optimization in order to improve effectiveness of drug delivery.

Figure-2: Flow-through diffusion cells

The model utilizes human cadaver nails mounted in flow-through diffusion cells. Following topical application of the onycomychotic drug formulation(s) at a clinically relevant exposure level, receptor medium is collected to gain an absorption profile for a drug that has passed through the nail. The nail is then sectioned longitudinally, providing distribution and kinetic information of the drug within the nail plate. The use of full-thickness human nails provides a realistic surrogate for *in vivo* absorption. The *in vitro* ungual absorption model lends itself well to formulation development and selection. For a drug intended to be transungually delivered, screening using many formulations may be performed, the end point of which will be total absorption. Lead candidate formulations can then be selected for mass balance studies. Flow-through systems can be adapted to include a semi-occlusive graphite filter for use with volatile test items to ensure maximum recoveries. These studies generally use a radiolabeled test item. If radiochemical is not available, then appropriate extraction and analytical methods for each of the matrices of interest are develop and validate. (In Vitro Sciences In Vitro Ungual (Nail) Absorption 2011)

Figure-2: Close-up of nail in diffusion cell following application of lacquer

Nail Lacquers As Transungual Drug Delivery Vehicles

Creams, ointments, gels, solutions, lotions, foams, pastes etc. are available but present trend is nail lacquers.

NAIL LACQERS: -

Medicated nail lacquers are the formulations that are used for transungual drug delivery system for maximal antifungal efficacy. After application, the solvent from the lacquer formulation evaporates leaving an occlusive film on which the drug concentration is higher than in the original formulation. This increases the diffusion gradient and permeation through dense keratinized nail plate occurs. The film on the nail surface acts as a drug “depot” that permits optimized and sustained diffusion across the nail and leads to continuous penetration of active principle to high tissue concentration required for the efficacy for the treatment of Onychomycosis.

Two factors may limit the accumulation and activity of drugs in the nail on topical application. The physicochemical properties of the drug need to be favorable for absorption through nail matrix. The nail matrix is relatively more permeable to polar compounds than non-polar compounds. Second, binding of the drug to keratin reduces the availability of the free drug. Antifungal drugs are reported to possess high binding affinity to keratin.

Chemical Penetration Enhancement

The common approach for enhancing nail drug delivery has been to use Keratolytic and thiolytic agents. These agents are known to increase the permeability of nail matrix by chemical modification of keratin. However, their permeability enhancement is limited by the factors like penetrability of enhancer and the duration of its presence in the nail matrix might significantly influence the chemical modification of keratin.

Physical Penetration Enhancement

Iontophoresis is the method of choice to enhance delivery of drug by physical method. Recently the

iontophoretic trans-nail delivery method showed good results in treating nail fungal syndromes. S. Narsimha Murthy and co-workers have studied. The effect of Iontophoresis on the permeability of salicylic acid across human nail plate has been studied. Diffusion study using Franz diffusion cell was conducted by incorporating an electrode with it .The results showed drastic increase in the permeability of a test penetrant across nail plate as compared with the conventional method of penetration. The penetration of antifungal agents through human nail can be studied by using Photoacoustic Spectroscopy. The main task is to identify whether the substances used as vehicles are efficient to carry the active substance to reach the nail bed. Photoacoustic Spectroscopy (PAS) can be used to as a valuable technique to evaluate the penetration of substances through human skin.
(R PATEL 2009) (Nuglisch L 2005)

Evaluation Parameters

Nonvolatile content

Pre-determined weight of sample is taken in a glass Petri dish of about. Samples are spread evenly with the help of tared wire. The dish is placed in the oven at 105°C ± 2°C for 1 hr. After 1 hr the Petri dish is removed, cooled, and weighed. The difference in weight of sample after drying is determined.

Drying Time

A film of sample is applied on a glass Petri dish with the help of brush. The time to form a dry-to-touch film is noted using a stopwatch.

Smoothness of flow

The sample is poured to approximately 1.5 inches and spread on a glass plate and made to rise vertically.

Gloss

Gloss of the film is visually seen, comparing it with a standard marketed nail lacquer. (S. Firoz 2011)

In vitro studies

Diffusion studies are performed using artificial membrane (Gelman Laboratory) of pore size 0.2 µm. The membrane is soaked for 1 h in solvent system A (phosphate buffer, pH 7.4; and methanol, AR grade, in the ratio of 4:1), and the receptor compartment is filled with solvent A. Test vehicle equivalent to 200 µg is applied evenly on the surface of the membrane. The prepared membrane is mounted on the cell carefully to avoid entrapment of air bubbles under the membrane. The whole assembly is maintained at 37°C, and the speed of stirring is kept constant (600 rpm) for 7 h. The 2-mL aliquot of drug sample is taken after a time interval of 1 h and replaced by the fresh solvent A. The experiment is performed in triplicate. The drug analysis is done using double-beam UV spectrophotometer; model V-530 (Jasco Corporation, Japan).

In vitro transungual permeation studies

Hooves from freshly slaughtered cattle, free of adhering connective and cartilaginous tissue, are soaked in distilled water for 24 h. Membranes of about 1-mm thickness are then cut from the distal part of hooves. *In vitro* permeation studies are carried out by using Franz diffusion cell (respective volume, 25 mL), the hoof membrane is placed carefully on the cell, and the surface area available for permeation was 1.23 cm^2 . Then the test vehicle equivalent to 200 µg is applied evenly on the surface of the nail membrane. The receptor compartment is filled with solvent A (phosphate buffer, pH 7.4; and methanol, AR grade, in the ratio of 4:1), and the whole assembly is maintained at 37°C with constant stirring (600 rpm) for 30 h. The 2-mL aliquot of drug sample is taken after a time interval of 1 h and is replaced by the fresh solvent A. The experiment is performed in triplicate. The drug analysis was done by using double-beam UV spectrophotometer; model V-530 (Jasco Corporation, Japan).

Recent Trends in Nail Drug Delivery

The traditional formulations like nail lacquers, nail varnish, and nail patches have been replaced by recent technologies. These are introduced in the development of more efficient drug delivery.

Electro chemotherapy for Nail disorders:-

The goal of this therapy is to develop an active method of drug delivery across the nail plate which in turn is believed to increase the success rate of topical monotherapy and decrease the duration of treatment of nail disorders. Currently, the electrically mediated techniques for drug delivery across the nail plate are investigated.

Transungual Iontophoresis:-

Iontophoresis is found to enhance the transport of drugs across the nail plate significantly. The predominant mechanisms contributing to enhanced transport of drugs in the case of trans nail iontophoresis are electrophoresis and electro osmosis. With proper Iontophoretic device, the transport of not only the ionic drugs but also uncharged drugs could be enhanced across the nail stratums. The transport of glucose and griseofulvin across the human nail indicates that the nail plate exhibits iontophoretic selectivity similar to human skin.

Mesoscissioning technology:-

Mesoscissioning technology creates a micro-conduit through the skin or nail within a specified depth range. Fully open pathways can be painlessly sized through the stratum corneum of the skin or through the nail. Micro conduits, 300-500 microns in diameter, are produced within seconds and without sensation. These pathways can be used to deliver drugs across the skin. In nails, micro conduits quickly reduce the painful pressure of subungual hematoma (black toe) and serve as a prophylactic to prevent pressure build-up.

Nano Patch Nail Fungus:-

Nano Patch Fungus uses AC/DC electrochemistry and targeted drug delivery to actively push antifungal drugs right through the nail cuticle to the actual location of the fungus growth. This technology targets nail fungus at its source of growth.

Laser therapy:-

Physical treatment of the nail such as laser therapy creates partial micro holes in the nail plate that allow better penetration of nail lacquers.

Hydration of the nail:-

The chemical composition of the nail plate indicates that the aqueous pathway plays the dominant role in drug penetration through the nail. Furthermore, water is the principle nail plasticizer. Once hydrated, the nail becomes more elastic and possibly more permeable to topically applied substances. (Dehghan M 2008), (R PATEL 2009)

The permeability of the compact, highly keratinized nail plate to topically applied drugs is poor and drug uptake into the nail apparatus is extremely low. Topical therapy is worth pursuing however, as local action is required in many nail disorders. Drug transport into the nail plate can be assisted by filing the nail plate before topical application of drug formulations as well as by the use of chemical enhancers. The future for manufacturers of drug-containing lacquers, especially of anti-fungal containing lacquers is bright, given the increasing prevalence of Onychomycosis, and a large number of patents covering anti-fungal nail lacquers have been filed. Future pharmaceutical lacquer formulations can include, ungual permeation enhancers, such as, oxacyclohexadecan-2-one, which could increase drug flux into the nail, keratolytic agents such as urea, salicylic acid, enzymes, which could increase drug flux into the nail acidified formulations.

Marketed Preparations:

Drug	Brand name	Company
Ciclopiroxamine 8%	Onylac	Cipla,India
Ciclopiroxamine 8%	Penlac	Dermik,Canada
Amorolfine 5%	Loceryl	Roche Lab, Australia
Ciclopiroxamine 8%	Nailon	Protech biosystem,India
Econazole 5%	Econail	Macrochem corporation, Lexington
Urea 40%	Umecta	JSJ Pharmaceuticals
Tazarotene	Tazorac 0.1% gel	Allergan Inc
Sertaconazol nitrate	Zalain nail patch	Labtec
Amorolfine 5 %	Curanail	Galderma

The drug concentration in the film is much higher than concentration in the original nail lacquer as the solvent evaporates and a film is formed. In addition, drug-containing lacquers must be colorless and non-glossy to be acceptable to male patients. Most importantly, the drug must be released from the film so that it can penetrate into the nail. Currently marketed topical therapies include Amorolfine (Loceryl®, Galderma) and Ciclopirox (Penlac®, Dermik). Penlac® is applied once daily for up to 48 weeks. However, the formulation is removed every 7 days with alcohol before reapplication. (Vivek R 2012)

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REGULATORY ASPECTS OF DRUG DELIVERY SYSTEMS

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Introduction

In recent years use of modified release drug formulation has increased considerably. This is partly due to advances in formulation technologies enabling one to design formulation that provide better control of the release of drug to the site of action.controlled release formulation provide several advantages over convention lmmediate-release formulations of same drug , such as redused dosing frequency, a decreased incidence and/or intensity of side effects, a greater selectivity of pharmacological activity, and reduction in drug, plasma fluctuation resulting in a more constant or prolonged therapeutic effect. (www.fda.gov n.d.)

However, MR formulation provide unique challenges from a formulation and manufacturing point of view requiring specific studies to characterize the controlled-release nature of the formation. IN this chapter the regulatory considerations that come into play in approving and maintaining such formation ion the market will be discussed with an emphasis on the laws, regulation, and guidances that pertain to this type of drug delivery formulation.

The requirements discussed in this chapter cover all type of controlled release dosage form.

The primary focus will be on oral controlled-release drug products, which are the most common type of controlled Release dosage form. Requirements for other types of controlled-release drug products, such as transdermal patches or implants, are similar to those described in this chapter.

Definitions

Before beginning a discussion of the regulatory requirement of controlled-release products it is to understand several commonly used definitions for these types of products.

Controlled release dosage forms: A class of pharmaceutical or other biologically active product from which a drug is released from the delivery system in a planned, predictable, and slower-than-normal manner.

Modified-release dosage form: Refers, in general, to a dosage form for which the drug release characteristics of time course and/or location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage form.

Extended-release dosage form: This is a specific type of MR dosage form that allows at least a twofold reduction in dosage frequency as compared to that drug presented as an immediate (conventional)-release dosage form (IR). (Code of federal REGULATION 21 320.25 n.d.)

Delayed-release dosage form: This is a specific type of MR dosage form that releases a drug at a time other than promptly after administration. An example is enteric-coated tablets.

Proportional similarity:

Definition 1: All active and inactive ingredients are in exactly the same proportion between different strengths (e.g. a tablet of 50-mg strength has all the inactive ingredients exactly half that of 100-mg strength and twice that of a tablet of 25-mg strength).

Definition 2: The total weight of the dosage form remains nearly the same for all strengths (within 5% of the total weight of the strength on which a biostudy was performed), the same inactive ingredients are used for all strength, and the changes in any strength is obtained by altering the amount of active ingredient. For example, with respective to approved 5-mg tablet, the total weight of new 1-and 2.50mg tablets remains nearly same, and the changes in the amount of active ingredient are offset by a change in one or more inactive ingredients. This definition is generally applicable to high-potency drug substances where the amount of active drug substance in the dosage form is relatively low.

Controlled release new drug applications

A fundamental question in evaluating a controlled-release product is whether formal clinical studies of the dosage form’s safety and efficacy are needed or whether only a pharmacokinetic evaluation will provide adequate evidence for approval. A rational answer to this question must be based on evaluation of the pharmacokinetic properties and plasma concentration/effect relationship of the drug. Where there is a well-defined predictive relationship between the plasma concentrations of the drug and the clinical response (regarding both safty and efficacy), it may be possible to rely on plasma concentration data alone as a basis for the approval of the controlled-release product. In the following situationst is expected that clinical safty and efficacy data be submitted for the approval of the controlled release new drug application (NDA):

- When the controlled-release product involves a drug that is an unapproved new molecular entity, since there is no approved reference product to which a bioequivalence claim could be made.
- When the rate of input has an effect on the drug's efficacy and toxicity profile.
- When a claim of therapeutic advantage is intended for the controlled-release product.
- When there are safety concerns with regard to irreversible toxicity.
- Where there are uncertainties concerning the relationship between plasma concentration and therapeutic and adverse effects or in the absence of a well-defined relationship between plasma concentration and either therapeutic or adverse clinical response.
- Where there is evidence of functional (i.e. pharmacodynamic) tolerance.
- Where peak-to-trough differences of the IR form are very large.
-

In all the above instances where there is already an IR formulation of the drug, a 505(b) (2) NDA could be submitted for approval to the Food and Drug Administration (FDA). The revolution for a 505(b)(2) NDA are covered under 21 CFR 314.54. These regulations state that any person seeking approval of a drug product that represents a modification of a listed drug, eg. a new indication or a new dosage form, and for which investigations other than bioavailability or bioequivalence studies are essential to the approval of the changes, may submit a 505(b) (2) application. However such an application may not be submitted under this section of the regulations for a drug product whose only difference from the reference listed drug is that the extent of absorption or rate of absorption is less than that of the reference listed drug or if the rate of absorption is unintentionally less than that of the reference listed drug.

Bioavailability and bioequivalence requirements

Subpart A- General Provisions

Sec. 320.1 Definitions

(a) Bioavailability means the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

(**b) Drug product** means a finished dosage form, e.g., tablet, capsule, or solution that contains the active drug ingredient, generally, but not necessarily, in association with inactive ingredients.

(c) Pharmaceutical equivalents means drug products in identical dosage forms that contain identical amounts of the identical active drug ingredient, i.e. , the same salt or ester of the same therapeutic moiety, or, in the case of modified release dosage forms that require a reservoir or overage or such forms as prefilled syringes where residual volume may vary, that deliver identical amounts of the active drug ingredient over the identical dosing period; do not necessarily contain the same inactive ingredients; and meet the identical compendial or other applicable standard of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times, and/or dissolution rates.

(d) Pharmaceutical alternatives means drug products that contain the identical therapeutic moiety, or its precursor, but not necessarily in the same amount or dosage form or as the same salt or ester. Each such drug product individually meets either the identical or its own respective compendial or other applicable standard of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times and/or dissolution rates.

(e) Bioequivalence means the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study. Where there is an intentional difference in rate (e.g., in certain extended release dosage forms), certain pharmaceutical equivalents or alternatives may be considered bioequivalent if there is no significant difference in the extent to which the active ingredient or moiety from each product becomes available at the site of drug action. This applies only if the difference in the rate at which the active ingredient or moiety becomes available at the site of drug action is intentional and is reflected in the proposed labeling, is not essential to the attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug.

(f) Bioequivalence requirement means a requirement imposed by the Food and Drug Administration for in vitro and/or in vivo testing of specified drug products which must be satisfied as a condition of marketing.

(g) Same drug product formulation means the formulation of the drug product submitted for approval and any formulations that have minor differences in composition or method of manufacture from the formulation submitted for approval, but are similar enough to be relevant to the agency's determination of bioequivalence (Code of Federal Regulation21 320.1 n.d.)

Subpart B- Procedures for Determining the Bioavailability or Bioequivalence of Drug Products

Sec. 320.21 Requirements for submission of bioavailability and bioequivalence data

(a) Any person submitting a full new drug application to the Food and Drug Administration (FDA) shall include in the application either:

(1) Evidence measuring the in vivo bioavailability of the drug product that is the subject of the application;

or

(2) Information to permit FDA to waive the submission of evidence measuring in vivo bioavailability.

(b) Any person submitting an abbreviated new drug application to FDA shall include in the application either:

(1) Evidence demonstrating that the drug product that is the subject of the abbreviated new drug application is bioequivalent to the reference listed drug (defined in 314.3(b) of this chapter). A complete study report must be submitted for the bioequivalence study upon which the applicant relies for approval. For all other bioequivalence studies conducted on the same drug product formulation, the applicant must submit either a complete or summary report. If a summary report of a bioequivalence study is submitted and FDA determines that there may be bioequivalence issues or concerns with the product, FDA may require that the applicant submit a complete report of the bioequivalence study to FDA; or

(2) Information to show that the drug product is bioequivalent to the reference listed drug which would permit FDA to waive the submission of evidence demonstrating in vivo bioequivalence as provided in paragraph (f) of this section.

(c) Any person submitting a supplemental application to FDA shall include in the supplemental application the evidence or information set forth in paragraphs (a) and (b) of this section if the supplemental application proposes any of the following changes:

(1) A change in the manufacturing site or a change in the manufacturing process, including a change in product formulation or dosage strength, beyond the variations provided for in the approved application.

(2) A change in the labeling to provide for a new indication for use of the drug product, if clinical studies are required to support the new indication for use.

(3) A change in the labeling to provide for a new dosage regimen or for an additional dosage regimen for a special patient population, e.g., infants, if clinical studies are required to support the new or additional dosage regimen.

(d) FDA may approve a full new drug application, or a supplemental application proposing any of the changes set forth in paragraph (c) of this section, that does not contain evidence of in vivo bioavailability or information to permit waiver of the requirement for in vivo bioavailability data, if all of the following conditions are met.

(1) The application is otherwise approvable.

(2) The application agrees to submit, within the time specified by FDA, either:

(i) Evidence measuring the in vivo bioavailability and demonstrating the in vivo bioequivalence of the drug product that is the subject of the application;

or

(ii) Information to permit FDA to waive measurement of in vivo bioavailability.

(e) Evidence measuring the in vivo bioavailability and demonstrating the in vivo bioequivalence of a drug product shall be obtained using one of the approaches for determining bioavailability set forth in 320.24.

(f) Information to permit FDA to waive the submission of evidence measuring the in vivo bioavailability or demonstrating the in vivo bioequivalence shall meet the criteria set forth in 320.22.

(g) Any person holding an approved full or abbreviated new drug application shall submit to FDA a supplemental application containing new evidence measuring the in vivo bioavailability or demonstrating the in vivo bioequivalence of the drug product that is the subject of the application if notified by FDA that:

(1) There are data demonstrating that the dosage regimen in the labeling is based on incorrect assumptions or facts regarding the pharmacokinetics of the drug product and that following this dosage regimen could potentially result in subtherapeutic or toxic levels;

Or

(2) There are data measuring significant intra-batch and batch-to-batch variability, e.g., plus or minus 25 percent, in the bioavailability of the drug product.

(h) The requirements of this section regarding the submission of evidence measuring the in vivo bioavailability or demonstrating the in vivo bioequivalence apply only to a full or abbreviated new drug application or a supplemental application for a finished dosage formulation.

Sec. 320.22 Criteria for waiver of evidence of in vivo bioavailability or bioequivalence

(a) Any person submitting a full or abbreviated new drug application, or a supplemental application proposing any of the changes set forth in 320.21(c), may request FDA to waive the requirement for the submission of evidence measuring the in vivo bioavailability or demonstrating the in vivo bioequivalence of the drug product that is the subject of the application. An applicant shall submit a request for waiver with the application. Except as provided in paragraph (f) of this section, FDA shall waive the requirement for the submission of evidence of in vivo bioavailability or bioequivalence if the drug product meets any of the provisions of paragraphs (b), (c), (d), or (e) of this section.

(b) For certain drug products, the in vivo bioavailability or bioequivalence of the drug product may be self-evident. FDA shall waive the requirement for the submission of evidence obtained in vivo measuring the bioavailability or demonstrating the bioequivalence of these drug products. A drug product's in vivo bioavailability or bioequivalence may be considered self-evident based on other data in the application if the product meets one of the following criteria:

(1)The drug product:

(i) Is a parenteral solution intended solely for administration by injection, or an ophthalmic or otic solution; and

(ii) Contains the same active and inactive ingredients in the same concentration as a drug product that is the subject of an approved full new drug application or abbreviated new drug application.

(2) The drug product:

(i) Is administered by inhalation as a gas, e.g., a medicinal or an inhalation anesthetic; and

(ii) Contains an active ingredient in the same dosage form as a drug product that is the subject of an approved full new drug application or abbreviated new drug application.

(3) The drug product:

(i) Is a solution for application to the skin, an oral solution, elixir, syrup, tincture, a solution for aerosolization or nebulization, a nasal solution, or similar other solubilized form; and

(ii) Contains an active drug ingredient in the same concentration and dosage form as a drug product that is the subject of an approved full new drug application or abbreviated new drug application; and

(iii) Contains no inactive ingredient or other change in formulation from the drug product that is the subject of the approved full new drug application or abbreviated new drug application that may significantly affect absorption of the active drug ingredient or active moiety for products that are systemically absorbed, or that may significantly affect systemic or local availability for products intended to act locally.

(c) FDA shall waive the requirement for the submission of evidence measuring the in vivo bioavailability or demonstrating the in vivo bioequivalence of a solid oral dosage form (other than a delayed release or extended release dosage form) of a drug product determined to be effective for at least one indication in a Drug Efficacy Study Implementation notice or which is identical, related, or similar to such a drug product under 310.6 of this chapter unless FDA has evaluated the drug product under the criteria set forth in 320.33, included the drug product in the Approved Drug Products with Therapeutic Equivalence Evaluations List, and rated the drug product as having a known or potential bioequivalence problem. A drug product so rated reflects a determination by FDA that an in vivo bioequivalence study is required.

(d) For certain drug products, bioavailability may be measured or bioequivalence may be demonstrated by evidence obtained in vitro in lieu of in vivo data. FDA shall waive the requirement for the submission of evidence obtained in vivo measuring the bioavailability or demonstrating the bioequivalence of the drug product if the drug product meets one of the following criteria:

(1) [Reserved]

(2) The drug product is in the same dosage form, but in a different strength, and is proportionally similar in its active and inactive ingredients to another drug product for which the same manufacturer has obtained approval and the conditions in paragraphs (d)(2)(i) through (d)(2)(iii) of this section are met:

(i) The bioavailability of this other drug product has been measured;

(ii) Both drug products meet an appropriate in vitro test approved by FDA; and

(iii) The applicant submits evidence showing that both drug products are proportionally similar in their active and inactive ingredients.

(iv) Paragraph (d) of this section does not apply to delayed release or extended release products.

(3) The drug product is, on the basis of scientific evidence submitted in the application, shown to meet an in vitro test that has been correlated with in vivo data.

(4) The drug product is a reformulated product that is identical, except for a different color, flavor, or preservative that could not affect the bioavailability of the reformulated product, to another drug product for which the same manufacturer has obtained approval and the following conditions are met:

(i) The bioavailability of the other product has been measured; and

(ii) Both drug products meet an appropriate in vitro test approved by FDA.

(e) FDA, for good cause, may waive a requirement for the submission of evidence of in vivo bioavailability or bioequivalence if waiver is compatible with the protection of the public health. For full new drug applications, FDA may defer a requirement for the submission of evidence of in vivo bioavailability if deferral is compatible with the protection of the public health.

(f) FDA, for good cause, may require evidence of in vivo bioavailability or bioequivalence for any drug product if the agency determines that any difference between the drug product and a listed drug may affect the bioavailability or bioequivalence of the drug product. (Code of Federal Regulation 21 320.22 n.d.)

Sec. 320.23 Basis for measuring in vivo bioavailability or demonstrating bioequivalence

(a) (1) The in vivo bioavailability of a drug product is measured if the product's rate and extent of absorption, as determined by comparison of measured parameters, e.g., concentration of the active drug ingredient in the blood, urinary excretion rates, or pharmacological effects, do not indicate a significant difference from the reference material's rate and extent of absorption. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

(2) Statistical techniques used shall be of sufficient sensitivity to detect differences in rate and extent of absorption that are not attributable to subject variability.

(3) A drug product that differs from the reference material in its rate of absorption, but not in its extent of absorption, may be considered to be bioavailable if the difference in the rate of absorption is intentional, is appropriately reflected in the labeling, is not essential to the attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug product.

(b) Two drug products will be considered bioequivalent drug products if they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose of the active moiety under similar experimental conditions, either single dose or multiple dose. Some pharmaceutical equivalents or pharmaceutical alternatives may be equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on chronic use, and are considered medically insignificant for the particular drug product studied. (Code of Federal Regulation 21 320.23 n.d.)

Sec. 320.24 Types of evidence to measure bioavailability or establish bioequivalence

(a) Bioavailability may be measured or bioequivalence may be demonstrated by several in vivo and in vitro methods. FDA may require in vivo or in vitro testing, or both, to measure the bioavailability of a drug product or establish the bioequivalence of specific drug products. Information on bioequivalence requirements for specific products is included in the current edition of FDA's publication "Approved Drug Products with Therapeutic Equivalence Evaluations" and any current supplement to the publication. The selection of the method used to meet an in vivo or in vitro testing requirement depends up on the purpose of the study, the analytical methods available, and the nature of the drug product. Applicants shall conduct bioavailability and bioequivalence testing using the most accurate, sensitive, and reproducible approach available among those set forth in paragraph (b) of this section. The method used must be capable of measuring bioavailability or establishing bioequivalence, as appropriate, for the product being tested.

(b) The following in vivo and in vitro approaches, in descending order of accuracy, sensitivity, and reproducibility, are acceptable for determining the bioavailability or bioequivalence of a drug product.

(1) (i) An in vivo test in humans in which the concentration of the active ingredient or active moiety, and, when appropriate, its active metabolite(s), in whole blood, plasma, serum, or other appropriate biological fluid is measured as a function of time. This approach is particularly applicable to dosage forms intended to deliver the active moiety to the bloodstream for systemic distribution within the body;

Or

(ii) An in vitro test that has been correlated with and is predictive of human in vivo bioavailability data; or

(2) An in vivo test in humans in which the urinary excretion of the active moiety, and, when appropriate, its active metabolite(s), are measured as a function of time. The intervals at which measurements are taken should ordinarily be as short as possible so that the measure of the rate of elimination is as accurate as possible. Depending on the nature of the drug product, this approach may be applicable to the category of dosage forms described in paragraph (b)(1)(i) of this section. This method is not appropriate where urinary excretion is not a significant mechanism of elimination.

(3) An in vivo test in humans in which an appropriate acute pharmacological effect of the active moiety, and, when appropriate, its active metabolite(s), are measured as a function of time if such effect can be measured with sufficient accuracy, sensitivity, and reproducibility. This approach is applicable to the category of dosage forms described in paragraph (b)(1)(i) of this section only when appropriate methods are not available for measurement of the concentration of the moiety, and, when appropriate, its active metabolite(s), in biological fluids or excretory products but a method is available for the measurement of an appropriate acute pharmacological effect. This approach may be particularly applicable to dosage forms that are not intended to deliver the active moiety to the bloodstream for systemic distribution.

(4) Well-controlled clinical trials that establish the safety and effectiveness of the drug product, for purposes of measuring bioavailability, or appropriately designed comparative clinical trials, for purposes of demonstrating bioequivalence. This approach is the least accurate, sensitive, and reproducible of the general approaches for measuring bioavailability or demonstrating bioequivalence. For dosage forms intended to deliver the active moiety to the bloodstream for systemic distribution, this approach may be considered acceptable only when analytical methods cannot be developed to permit use of one of the approaches outlined in paragraphs (b)(1)(i) and (b)(2) of this section, when the approaches described in paragraphs (b)(1)(ii), (b)(1)(iii), and (b)(3) of this section are not available. This approach may also be considered sufficiently accurate for measuring bioavailability or demonstrating bioequivalence of dosage forms intended to deliver the active moiety locally, e.g., topical preparations for the skin, eye, and mucous membranes; oral dosage forms not intended to be absorbed, e.g., an antacid or radiopaque medium; and bronchodilators administered by inhalation if the onset and duration of pharmacological activity are defined.

(5) A currently available in vitro test acceptable to FDA (usually a dissolution rate test) that ensures human in vivo bioavailability.

(6) Any other approach deemed adequate by FDA to measure bioavailability or establish bioequivalence.

(c) FDA may, notwithstanding prior requirements for measuring bioavailability or establishing bioequivalence, require in vivo testing in humans of a product at any time if the agency has evidence that the product:

(1) May not produce therapeutic effects comparable to a pharmaceutical equivalent or alternative with which it is intended to be used interchangeably;

(2) May not be bioequivalent to a pharmaceutical equivalent or alternative with which it is intended to be used interchangeably; or

(3) Has greater than anticipated potential toxicity related to pharmacokinetic or other characteristics. (Code of Federal Regulation 21 320.24 n.d.)

Sec. 320.25 Guidelines for the conduct of an in vivo bioavailability study

(a) Guiding principles.
(1) The basic principle in an in vivo bioavailability study is that no unnecessary human research should be done.

(2) An in vivo bioavailability study is generally done in a normal adult population under standardized conditions. In some situations, an in vivo bioavailability study in humans may preferably and more properly be done in suitable patients. Critically ill patients shall not be included in an in vivo bioavailability study unless the attending physician determines that there is a potential benefit to the patient.

(b) Basic design. The basic design of an in vivo bioavailability study is determined by the following:

(1) The scientific questions to be answered.

(2) The nature of the reference material and the dosage form to be tested.

(3) The availability of analytical methods.

(4) Benefit-risk considerations in regard to testing in humans.

(c) Comparison to a reference material. In vivo bioavailability testing of a drug product shall be in comparison to an appropriate reference material unless some other approach is more appropriate for valid scientific reasons.

(d) Previously unmarketed active drug ingredients or therapeutic moieties.

(1) An in vivo bioavailability study involving a drug product containing an active drug ingredient or therapeutic moiety that has not been approved for marketing can be used to measure the following pharmacokinetic data:

(i) The bioavailability of the formulation proposed for marketing; and

(ii) The essential pharmacokinetic characteristics of the active drug ingredient or therapeutic moiety, such as the rate of absorption, the extent of absorption, the half-life of the therapeutic moiety in vivo, and the rate of excretion and/or metabolism. Dose proportionality of the active drug ingredient or the therapeutic moiety needs to be established after single-dose administration and in certain instances after multiple-dose administration. This characterization is a necessary part of the investigation of the drug to support drug labeling.

(2) The reference material in such a bioavailability study should be a solution or suspension containing the same quantity of the active drug ingredient or therapeutic moiety as the formulation proposed for marketing.

(3) The reference material should be administered by the same route as the formulation proposed for marketing unless an alternative or additional route is necessary to answer the scientific question under study. For example, in the case of an active drug ingredient or therapeutic moiety that is poorly absorbed after oral administration, it may be necessary to compare the oral dosage form proposed

for marketing with the active drug ingredient or therapeutic moiety administered in solution both orally and intravenously.

(e)New formulations of active drug ingredients or therapeutic moieties approved for marketing.

(1) An in vivo bioavailability study involving a drug product that is a new dosage form, or a new salt or ester of an active drug ingredient or therapeutic moiety that has been approved for marketing can be used to:

(i) Measure the bioavailability of the new formulation, new dosage form, or new salt or ester relative to an appropriate reference material; and

(ii) Define the pharmacokinetic parameters of the new formulation, new dosage form, or new salt or ester to establish dosage recommendation.

(2) The selection of the reference material(s) in such a bioavailability study depends upon the scientific questions to be answered, the data needed to establish comparability to a currently marketed drug product, and the data needed to establish dosage recommendations.

(3) The reference material should be taken from a current batch of a drug product that is the subject of an approved new drug application and that contains the same active drug ingredient or therapeutic moiety, if the new formulation, new dosage form,or new salt or ester is intended to be comparable to or to meet any comparative labeling claims made in relation to the drug product that is the subject of an approved new drug application.

(f)Extended release formulations.
(1) The purpose of an in vivo bioavailability study involving a drug product for which an extended release claim is made is to determine if all of the following conditions are met:

(i) The drug product meets the extended release claims made for it.

(ii) The bioavailability profile established for the drug product rules out the occurrence of any dose dumping.

(iii) The drug product's steady-state performance is equivalent to a currently marketed nonextended release or extended release drug product that contains the same active drug ingredient or therapeutic moiety and that is subject to an approved full new drug application.

(iv) The drug product's formulation provides consistent pharmacokinetic performance between individual dosage units.

(2) The reference material(s) for such a bioavailability study shall be chosen to permit an appropriate scientific evaluation of the extended release claims made for the drug product. The reference material shall be one of the following or any combination thereof:

(i) A solution or suspension of the active drug ingredient or therapeutic moiety.

(ii) A currently marketed noncontrolled release drug product containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling of the noncontrolled release drug product.

(iii) A currently marketed extended release drug product subject to an approved full new drug application containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling proposed for the extended release drug product.

(iv) A reference material other than one set forth in paragraph (f) (2) (i), (ii) or (iii) of this section that is appropriate for valid scientific reasons.

(g) Combination drug products.
(1) Generally, the purpose of an in vivo bioavailability study involving a combination drug product is to determine if the rate and extent of absorption of each active drug ingredient or therapeutic moiety in the combination drug product is

equivalent to the rate and extent of absorption of each active drug ingredient or therapeutic moiety administered concurrently in separate single-ingredient preparations.

(2) The reference material in such a bioavailability study should be two or more currently marketed, single-ingredient drug products each of which contains one of the active drug ingredients or therapeutic moieties in the combination drug product. The Food and Drug Administration may, for valid scientific reasons, specify that the reference material shall be a combination drug product that is the subject of an approved new drug application.

(3) The Food and Drug Administration may permit a bioavailability study involving a combination drug product to determine the rate and extent of absorption of selected, but not all, active drug ingredients or therapeutic moieties in the combination drug product. The Food and Drug Administration may permit this determination if the pharmacokinetics and the interactions of the active drug ingredients or therapeutic moieties in the combination drug product are well known and the therapeutic activity of the combination drug product is generally recognized to reside in only one of the active drug ingredients or therapeutic moieties, e.g., ampicillin in an ampicillin-probenecid combination drug product.

(h) Use of a placebo as the reference material. Where appropriate or where necessary to demonstrate the sensitivity of the test, the reference material in a bioavailability study may be a placebo if:

(1) The study measures the therapeutic or acute pharmacological effect of the active drug ingredient or therapeutic moiety; or

(2) The study is a clinical trial to establish the safety and effectiveness of the drug product.

(i) Standards for test drug product and reference material.

(1) Both the drug product to be tested and the reference material, if it is another drug product, shall be shown to meet all compendial or other applicable standards of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times, and dissolution rates.

(2) Samples of the drug product to be tested shall be manufactured using the same equipment and under the same conditions as those used for full-scale production. (Code of Federal Regulation 21 320.25 n.d.)

Sec. 320.26 Guidelines on the design of a single-dose in vivo bioavailability or bioequivalence study

(a) Basic principles.

(1) An in vivo bioavailability or bioequivalence study should be a single-dose comparison of the drug product to be tested and the appropriate reference material conducted in normal adults.

(2) The test product and the reference material should be administered to subjects in the fasting state, unless some other approach is more appropriate for valid scientific reasons.

(b) Study design.

(1) A single-dose study should be crossover in design, unless a parallel design or other design is more appropriate for valid scientific reasons, and should provide for a drug elimination period.

(2) Unless some other approach is appropriate for valid scientific reasons, the drug elimination period should be either:

(i) At least three times the half-life of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured in the blood or urine; or

(ii) At least three times the half-life of decay of the acute pharmacological effect.

(c) Collection of blood samples.

(1) When comparison of the test product and the reference material is to be based on blood

concentration time curves, unless some other approach is more appropriate for valid scientific reasons, blood samples should be taken with sufficient frequency to permit an estimate of both:

(i) The peak concentration in the blood of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured; and

(ii) The total area under the curve for a time period at least three times the half-life of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured.

(2) In a study comparing oral dosage forms, the sampling times should be identical.

(3) In a study comparing an intravenous dosage form and an oral dosage form, the sampling times should be those needed to describe both:

(i) The distribution and elimination phase of the intravenous dosage form; and

(ii) The absorption and elimination phase of the oral dosage form.

(4) In a study comparing drug delivery systems other than oral or intravenous dosage forms with an appropriate reference standard, the sampling times should be based on valid scientific reasons.

(d) Collection of urine samples. When comparison of the test product and the reference material is to be based on cumulative urinary excretion-time curves, unless some other approach is more appropriate for valid scientific reasons, samples of the urine should be collected with sufficient frequency to permit an estimate of the rate and extent of urinary excretion of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured.

(e) Measurement of an acute pharmacological effect.

(1) When comparison of the test product and the reference material is to be based on acute pharmacological effect-time curves, measurements of this effect should be made with sufficient frequency to permit a reasonable estimate of the total area under the curve for a time period at least three times the half-life of decay of the pharmacological effect, unless some other approach is more appropriate for valid scientific reasons.

(2) The use of an acute pharmacological effect to determine bioavailability may further require demonstration of dose-related response. In such a case, bioavailability may be determined by comparison of the dose-response curves as well as the total area under the acute pharmacological effect-time curves for any given dose.

Sec. 320.27 Guidelines on the design of a multiple-dose in vivo bioavailability study

(a) Basic principles.

(1) In selected circumstances it may be necessary for the test product and the reference material to be compared after repeated administration to determine steady-state levels of the active drug ingredient or therapeutic moiety in the body.

(2) The test product and the reference material should be administered to subjects in the fasting or nonfasting state, depending upon the conditions reflected in the proposed labeling of the test product.

(3) A multiple-dose study may be required to determine the bioavailability of a drug product in the following circumstances:

(i) There is a difference in the rate of absorption but not in the extent of absorption.

(ii) There is excessive variability in bioavailability from subject to subject.

(iii) The concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), in the blood resulting from a single dose is too low for accurate determination by the analytical method.

(iv) The drug product is an extended release dosage form.

(b) Study design.

(1) A multiple-dose study should be crossover in design, unless a parallel design or other design is more appropriate for valid scientific reasons, and should provide for a drug elimination period if steady-state conditions are not achieved.

(2) A multiple-dose study is not required to be of crossover design if the study is to establish dose proportionality under a multiple-dose regimen or to establish the pharmacokinetic profile of a new drug product, a new drug delivery system, or an extended release dosage form.

(3) If a drug elimination period is required, unless some other approach is more appropriate for valid scientific reasons, the drug elimination period should be either:

(i) At least five times the half-life of the active drug ingredient or therapeutic moiety, or its active metabolite(s), measured in the blood or urine; or

(ii) At least five times the half-life of decay of the acute pharmacological effect.

(c) Achievement of steady-state conditions. Whenever a multiple-dose study is conducted, unless some other approach is more appropriate for valid scientific reasons, sufficient doses of the test product and reference material should be administered in accordance with the labeling to achieve steady-state conditions.

(d) Collection of blood or urine samples.

(1) Whenever comparison of the test product and the reference material is to be based on blood concentration-time curves at steady state, appropriate dosage administration and sampling should be carried out to document attainment of steady state.

(2) Whenever comparison of the test product and the reference material is to be based on cumulative urinary excretion-time curves at steady state, appropriate dosage administration and sampling should be carried out to document attainment of steady state.

(3) A more complete characterization of the blood concentration or urinary excretion rate during the absorption and elimination phases of a single dose administered at steady-state is encouraged to permit estimation of the total area under concentration-time curves or cumulative urinary excretion-time curves and to obtain pharmacokinetic information, e.g., half-life or blood clearance, that is essential in preparing adequate labeling for the drug product.

(e) Steady-state parameters.

(1) In certain instances, e.g., in a study involving a new drug entity, blood clearances at steady-state obtained in a multiple-dose study should be compared to blood clearances obtained in a single-dose study to support adequate dosage recommendations.

(2) In a linear system, the area under the blood concentration-time curve during a dosing interval in a multiple-dose steady-state study is directly proportional to the fraction of the dose absorbed and is equal to the corresponding "zero to infinity" area under the curve for a single-dose study. Therefore, when steady-state conditions are achieved, a comparison of blood concentrations during a dosing interval may be used to define the fraction of the active drug ingredient or therapeutic moiety absorbed.

(3) Other methods based on valid scientific reasons should be used to determine the bioavailability of a drug product having dose-dependent kinetics (non-linear system).

(f) Measurement of an acute pharmacological effect. When comparison of the test product and the reference material is to be based on acute pharmacological effect-time curves, measurements of this effect should be made with sufficient frequency to demonstrate a maximum effect and a lack of significant difference between the test product and the reference material. (Code of Federal Regulation 21 320.27 n.d.)

Sec. 320.28 Correlation of bioavailability with an acute pharmacological effect or clinical evidence

Correlation of in vivo bioavailability data with an acute pharmacological effect or clinical evidence of safety and effectiveness may be required if needed to establish the clinical significance of a special claim, e.g., in the case of an extended release preparation. (Code of Federal Regulation 21 n.d.)

Sec. 320.29 Analytical methods for an in vivo bioavailability or bioequivalence study

(a) The analytical method used in an in vivo bioavailability or bioequivalence study to measure the concentration of the active drug ingredient or therapeutic moiety, or its active metabolite(s), in body fluids or excretory products, or the method used to measure an acute pharmacological effect shall be demonstrated to be accurate and of sufficient sensitivity to measure, with appropriate precision, the actual concentration of the active drug ingredient or therapeutic moiety, or its active metabolite(s), achieved in the body.

(b) When the analytical method is not sensitive enough to measure accurately the concentration of the active drug ingredient or therapeutic moiety, or its active metabolite(s), in body fluids or excretory products produced by a single dose of the test product, two or more single doses may be given together to produce higher concentration if the requirements of 320.31 are met (Code of Federal Regulation 21 n.d.)

Sec. 320.30 Inquiries regarding bioavailability and bioequivalence requirements and review of protocols by the Food and Drug Administration

(a) The Commissioner of Food and Drugs strongly recommends that, to avoid the conduct of an improper study and unnecessary human research, any person planning to conduct a bioavailability or bioequivalence study submit the proposed protocol for the study to FDA for review prior to the initiation of the study.

(b) FDA may review a proposed protocol for a bioavailability or bioequivalence study and will offer advice with respect to whether the following conditions are met:

(1) The design of the proposed bioavailability or bioequivalence study is appropriate.

(2) The reference material to be used in the bioavailability or bioequivalence study is appropriate.

(3) The proposed chemical and statistical analytical methods are adequate.

(c) (1) General inquiries relating to in vivo bioavailability requirements and methodology shall be submitted to the Food and Drug Administration, Center for Drug Evaluation and Research, Office of Clinical Pharmacology.

(2) General inquiries relating to bioequivalence requirements and methodology shall be submitted to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Bioequivalence. (Code of Federal Regulation 21 n.d.)

Sec. 320.31 Applicability of requirements regarding an "Investigational New Drug Application."

(a) Any person planning to conduct an in vivo bioavailability or bioequivalence study in humans shall submit an "Investigational New Drug Application" (IND) if:

(1) The test product contains a new chemical entity as defined in 314.108(a) of this chapter; or

(2) The study involves a radioactively labeled drug product; or

(3) The study involves a cytotoxic drug product.

(b) Any person planning to conduct a bioavailability or bioequivalence study in humans using a drug product that contains an already approved, non-new chemical entity shall submit an IND if the study is one of the following:

(1) A single-dose study in normal subjects or patients where either the maximum single or total daily dose exceeds that specified in the labeling of the drug product that is the subject of an approved new drug application or abbreviated new drug application.

(2) A multiple-dose study in normal subjects or patients where either the single or total daily dose exceeds that specified in the labeling of the drug product that is the subject of an approved new drug application or abbreviated new drug application.

(3) A multiple-dose study on an extended release product on which no single-dose study has been completed.

(c) The provisions of parts 50, 56, and 312 of this chapter are applicable to any bioavailability or bioequivalence study in humans conducted under an IND.

(d) A bioavailability or bioequivalence study in humans other than one described in paragraphs (a) through (c) of this section is exempt from the requirements of part 312 of this chapter if the following conditions are satisfied:

(1) If the study is one described under 320.38(b) or 320.63, the person conducting the study, including any contract research rganization, shall retain reserve samples of any test article and reference standard used in the study and release the reserve samples to FDA upon request, in accordance with, and for the period specified in, 320.38; and

(2) An in vivo bioavailability or bioequivalence studyin humans shall be conducted in compliance with the requirements for institutional review set forth in part 56 of this chapter, and informed consent set forth in part 50 of this chapter. (Code of Federal Regulation 21 n.d.)

Sec. 320.32 Procedures for establishing or amending a bioequivalence requirement

(a) The Food and Drug Administration, on its own initiative or in response to a petition by an interested person, may propose and promulgate a regulation to establish a bioequivalence requirement for a product not subject to section 505(j) of the act if it finds there is well-documented evidence that specific pharmaceutical equivalents or pharmaceutical alternatives intended to be used interchangeably for the same therapeutic effect:

(1) Are not bioequivalent drug products; or

(2) May not be bioequivalent drug products based on the criteria set forth in 320.33; or

(3) May not be bioequivalent drug products because they are members of a class of drug products that have close structural similarity and similar physicochemical or pharmacokinetic properties to other drug products in the same class that FDA finds are not bioequivalent drug products.

(b) FDA shall include in a proposed rule to establish a bioequivalence requirement the evidence and criteria set forth in 320.33 that are to be considered in determining whether to issue the proposal. If the rulemaking is proposed in response to a petition, FDA shall include in the proposal a summary and analysis of the relevant information that was submitted in the petition as well as other available information to support the establishment of a bioequivalence requirement.

(c) FDA, on its own initiative or in response to a petition by an interested person, may propose and promulgate an amendment to a bioequivalence requirement established under this subpart. (Code of Federal Regulation 21 n.d.)

Sec. 320.33 Criteria and evidence to assess actual or potential bioequivalence problems

The Commissioner of Food and Drugs shall consider the following factors, when supported by well-documented evidence, to identify specific pharmaceutical equivalents and pharmaceutical alternatives that are not or may not be bioequivalent drug products.

(a) Evidence from well-controlled clinical trials or controlled observations in patients that such drug products do not give comparable therapeutic effects.

(b) Evidence from well-controlled bioequivalence studies that such products are not bioequivalent drug products.

(c) Evidence that the drug products exhibit a narrow therapeutic ratio, e.g., there is less than a 2-fold difference in median lethal dose (LD50) and median effective dose (ED50) values, or have less than a 2-fold difference in the minimum toxic concentrations and minimum effective concentrations in the blood, and safe and effective use of the drug products requires careful dosage titration and patient monitoring.

(d) Competent medical determination that a lack of bioequivalence would have a serious adverse effect in the treatment or prevention of a serious disease or condition.

(e) Physicochemical evidence that:

(1) The active drug ingredient has a low solubility in water, e.g., less than 5 milligrams per 1 milliliter, or, if dissolution in the stomach is critical to absorption, the volume of gastric fluids required to dissolve the recommended dose far exceeds the volume of fluids present in the stomach (taken to be 100 milliliters for adults and prorated for infants and children).

(2) The dissolution rate of one or more such products is slow, e.g., less than 50 percent in 30 minutes when tested using either a general method specified in an official compendium or a paddle method at 50 revolutions per minute in 900 milliliters of distilled or deionized water at 37deg. C, or differs significantly from that of an appropriate reference material such as an identical drug product that is the subject of an approved full new drug application.

(3) The particle size and/or surface area of the active drug ingredient is critical in determining its bioavailability.

(4) Certain physical structural characteristics of the active drug ingredient, e.g., polymorphic forms, conforms, solvates, complexes, and crystal modifications, dissolve poorly and this poor dissolution may affect absorption.

(5) Such drug products have a high ratio of excipients to active ingredients, e.g., greater than 5 to 1.

(6) Specific inactive ingredients, e.g., hydrophilic or hydrophobic excipients and lubricants, either may be required for absorption of the active drug ingredient or therapeutic moiety or, alternatively, if present, may interfere with such absorption.

(f) Pharmacokinetic evidence that:

(1) The active drug ingredient, therapeutic moiety, or its precursor is absorbed in large part in a particular segment of the gastrointestinal tract or is absorbed from a localized site.

(2) The degree of absorption of the active drug ingredient, therapeutic moiety, or its precursor is poor, e.g., less than 50 percent, ordinarily in comparison to an intravenous dose, even when it is administered in pure form, e.g., in solution.

(3) There is rapid metabolism of the therapeutic moiety in the intestinal wall or liver during the process of absorption (first-class metabolism) so the therapeutic effect and/or toxicity of such drug product is determined by the rate as well as the degree of absorption.

(4) The therapeutic moiety is rapidly metabolized or excreted so that rapid dissolution and absorption are required for effectiveness.

(5) The active drug ingredient or therapeutic moiety is unstable in specific portions of the gastrointestinal tract and requires special coatings or formulations, e.g., buffers, enteric coatings, and film coatings, to assure adequate absorption.

(6) The drug product is subject to dose dependent kinetics in or near the therapeutic range, and the rate

and extent of absorption are important to bioequivalence. (Code of Federal Regulation 21 n.d.)

Sec. 320.34 Requirements for batch testing and certification by the Food and Drug Administration.

(a) If the Commissioner determines that individual batch testing by the Food and Drug Administration is necessary to assure that all batches of the same drug product meet an appropriate in vitro test, he shall include in the bioequivalence requirement a requirement for manufacturers to submit samples of each batch to the Food and Drug Administration and to withhold distribution of the batch until notified by the Food and Drug Administration that the batch may be introduced into interstate commerce.

(b) The Commissioner will ordinarily terminate a requirement for a manufacturer to submit samples for batch testing on a finding that the manufacturer has produced four consecutive batches that were tested by the Food and Drug Administration and found to meet the bioequivalence requirement, unless the public health requires that batch testing be extended to additional batches.

Sec. 320.35 Requirements for in vitro testing of each batch.

If a bioequivalence requirement specifies a currently available in vitro test or an in vitro bioequivalence standard comparing the drug product to a reference standard, the manufacturer shall conduct the test on a sample of each batch of the drug product to assure batch-to-batch uniformity.

Sec. 320.35 Requirements for in vitro testing of each batch.

If a bioequivalence requirement specifies a currently available in vitro test or an in vitro bioequivalence standard comparing the drug product to a reference standard, the manufacturer shall conduct the test on a sample of each batch of the drug product to assure batch-to-batch uniformity. (Code of Federal Regulation 21 n.d.)

Sec. 320.36 Requirements for maintenance of records of bioequivalence testing.

(a) All records of in vivo or in vitro tests conducted on any marketed batch of a drug product to assure that the product meets a bioequivalence requirement shall be maintained by the manufacturer for at least 2 years after the expiration date of the batch and submitted to the Food and Drug Administration on request.

(b) Any person who contracts with another party to conduct a bioequivalence study from which the data are intended to be submitted to FDA as part of an application submitted under part 314 of this chapter shall obtain from the person conducting the study sufficient accurate financial information to allow the submission of complete and accurate financial certifications or disclosure statements required under part 54 of this chapter and shall maintain that information and all records relating to the compensation given for that study and all other financial interest information required under part 54 of this chapter for 2 years after the date of approval of the application. The person maintaining these records shall, upon request for any properly authorized officer or employee of the Food and Drug Administration, at reasonable time, permit such officer or employee to have access to and copy and verify these records. (Code of Federal Regulation 21 n.d.)

Sec. 320.63 Retention of bioequivalence samples.

The applicant of an abbreviated application or a supplemental application submitted under section 505 of the Federal Food, Drug, and Cosmetic Act, or, if bioequivalence testing was performed under contract, the contract research organization shall retain reserve samples of any test article and reference standard used in conducting an in vivo or in vitro bioequivalence study required for approval of the abbreviated application or supplemental application. The applicant or contract research organization shall retain the reserve samples in accordance with, and for the period specified in, 320.38 and shall release the reserve samples to FDA upon request in accordance with 320.38. (Code of Federal Regulation 21 n.d.)

GENERAL APPROACHES FOR EVALUATING CONTROLED RELEASE PRODUCTS:

A. DEMONSTRATION OF SAFETY AND EFFICACY PRIMARILY BASED ON CLINICAL TRIALS:

In general for drugs where the exposure response has not been established or is unknown, application for changing the formulation from IR to MR will require demonstration of the safety and efficacy of the product in the target patient population. Typically the approval of such applications will be based on the results of the pivotal clinical trials (atleast two trials that are deemed pivotal to the assessment of the drug product from a clinical point of view).

In this case, the pharmacokinetics and biopharmaceutics studies conducted are for descriptive purposes and in certain cases will help in initial dose selection. The types of pharmacokinetic studies generally include:

1. Single dose relative bioavailability
2. Multiple dose relative bioavailability
3. Single dose bioequivalence studies
4. Dosage strength propornality
5. Food effect study
6. In-vivo in-vitro correlation (IVIVC)
7. Dose proportionality studies
8. Pharmacokinetic pharmacodynamic (PK/PD) evaluation

When a new molecular entity is developed as a MR dosage form, additional studies to characterize its clinical pharmacology and absorption distribution metabolism and excretion (ADME) characteristics are recommended. (Marrous 1997)

1. Example of an NDA with clinical data

The NDA for once-a-day formulation of diltiazem a typical example of an application with clinical data. The clinical portion of NDA consisted of three clinical trials. The trial was a randomized, double-blind placebo run in parallel-group pilot study with 36 patients with mild to moderate hypertension (24 on diltiazem 360 mg and 12 on placebo). The purpose of trial was to investigate the time-effect relationship of the diltiazem formulation. The second trial was considered to be one of the two pivotal trials and was a dose response trial in patients with mild to moderate hypertension. This was a multi center, randomized, double-blind, placebo-controlled, fixed dose response trial investigating 90, 180, 360, and 540 mg once a day diltiazem and placebo. A total of 229 patients participated in the study, which consisted of a four-week run-in phase followed by a 4-week active treatment period. The third trial (the second pivotal trial) was a multi centered dose titration trial for the treatment of mild to moderate hypertension. The trial was a multicentered, randomized, double-blind placebo controlled parallel study comparing optimally titrated doses ranging from 120 to 360 mg of diltiazem to placebo in a total of 117 patients.

The biopharmaceutics and clinical pharmacology portion of NDA consisted of 4 studies.

A single dose, relative bioavailability study comparing the controlled release formulation to he approved IR formulation.

A pivotal steady-state relative bioavailability and dose proportionality study.

A food effect and absorption profile study.

The sponsor also conducted a pilot relative bioavailability study to select the formulation with optimal release characteristics and also used the data obtained from this study to develop a multiple level C IVIVC.

The approval was mainly based on the results of the safety and efficacy trials where the biopharmaceutics study were undertaken to characterize the release properties of the formulation and ensure the no dose dumping is occurring. (Marroum 1997)

B. Demonstration of safety and efficacy based on PK, PK/PD trials

The "guidance for providing clinical evidence of effectiveness for human drug and biological products" states:

Sometimes clinical efficacy of modified-release dosage formulations can be extrapolated from existing studies, without the need for additional well-controlled clinical trials because other type of data allow the application of known effectiveness to the new dosage form. Even in the cases where blood levels are quite different, if there is a well-understood relationship between blood concentration and response, including an understanding of the time course of that relationship, it may be possible to conclude that the new dosage form is effective on the basis of pharmacokinetic data without an additional clinical efficacy trial.

The types of studies and requirement will depend on nature of exposure-response relationship and whether the therapeutic window is defined, as outlined below.

1. **There Is No Prior Knowledge Of The Exposure-Respense Relationship Or Therapeutic Window; Approval Is Solely Based On Plasma Concentrations.**

Such an approach, although being used in developing an MR product, is not encouraged. In such a case strict bioequivalence between the IR and MR product is required in terms of C_{max}, C_{min} and AUC at steady state.

The impact of differences in the shapes of the plasma concentration time profile for the IR and MR products should be assessed depending on knowledge of the drug, the therapeutic class, and the proposed indication for the drug. (Marroum 1997)

In certain instances, an MR product may be developed to actually mimic the performance of an IR product and its dosing regimen (e.g., Repetabs). The MR product is designed to simulate actual multiple single-dose administrations that would correspond to individual dosage administration of the IR product. Under such circumstances, with or without PK/PD information, it is conceivable that approval of the MR product could be based strictly on bioequivalence determination for the PK parameters. When deviations in the steady-state PK profiles are seen between the MR and IR product regimens, more dependence on PK/PD information or clinical studies would be required for approval rather than on simple bioequivalence of the pharmacokinetic parameters of AUC, C_{max} and C_{min}. (Marroum 1997)

2. **There Is No Quantitative Exposure-Response Relationship But A Well-Defined Therapeutic Window In Terms Of Safety And Efficacy**

In the case where the rate of input in known not to influence the safety and efficacy profile, the following criteria have to be met for the approval of such a product.

The 90% confidence interval for the log-transformed ratio of the AUC $_{ss}$ of the controlled release formulation relative to the IR-approved formulation should be between 80 and 125 (where as = steady state).

The $C_{max,ss}$ should be equal to or below the upper limit of the defined therapeutic window.

The C_{maxss} should be equal to or above the lower limit of the defined therapeutic window.

In the case where the rate of input is known ti influence the safety and efficacy profile or is unknown, the approval criteria are the same as above. In addition, studies investigating the impact of the rate of input on the pharmacodynamics of the drug in terms of safety and efficacy should be investigated. (Marroum 1997)

3. **There is a well defined quantitative exposure response relationship shown using different input rates of IR or the MR product**

Under such circumstances where an adequate efficacy and safety PK/PD relationship exists, further safety and efficacy studies may not be required. The exposure-response relationship should be established with the intended clinical end point. The safety profile of the drug shold be well understood. In such situations, steady state comparative BA/BE study would be required to demonstrate that the MR product that performs in a manner that ensures safety and efficacy under labeled dosing conditions. In addition, the standard PK/BA studies would also be required for descriptive and labeling purpose.

Under circumstances where clinical exposure or surrogates of such responses related to safety or efficacy have been preliminarily related to PK parameters or dose, further safety or efficacy studiesmay be required to confirm the preliminary PK/PD relationships. It is anticipated that such additional safety of efficacy studies would not be of the same scale where no preliminary PK/PD relationships had been shown. The standard PK/BA studies would also be required for descriptive and labeling purposes in such situations.

If the exposure-response relationship is established with a validated surrogate end point, the surrogate end point used should be accepted as a validated marker for clinical efficacy. In addition, the safety profile of the drug should be well understood. (Marroum 1997)

General considerations in evaluating pk/pd relationships for controlled-release drug products

In assessing PK/PD relationships for controlled-release products,it is important not only to establish concentration-effect relationships,but also to determine the significance of differences in the shape of the steady state concentration versus time profile for an MR product regimen as compared to the approved IR product regimen.In this regard,any differential effects based on the rate of absorption and/or the fluctuation within a profile as related to safety and/or efficacy should be determind.Issues of tolerance to therapeutic effects and toxic effect related to drug exposure,concentration,absorption rate,and fluctuation should also be examined as part of thePK/PD assessment.

In certain cases minimizing fluctuation in a steady-state profile for an MR product may be desirable to reduce toxicity,while maintaining efficacy as compared to the IR product regimen's profile where higher fluctuation is observed.it is therefore important and necessary to know or study the profile shape versus PD relationships.commenly made assumptions regarding therapeutic superiority or equivalency through fluctuation minimization in an MR product regimen versus an IR product regimen must be verified . (Marroum 1997)

Generic equivalence of an approved controlled release product

The drug price competition and patent term restoration act amendments , of 1984 , to the food rdug , and cosmetic act gave the FDA statutory authority o accept , and approve for marketing , abbreviated new drug applications for generic substitutes of pioneer products including those approved after 1962.To gain approval according to the law , ANDAs for generic controlled release drug product must , among other things , be both pharmaceutically equivalent and bioequivalent to the innovator controlled release product , which is term the reference listed drug producsame t as identified in FDAs approved drug product with therapeutic equivalence ratings (the orange book) (Marroum 1997)

Pharmaceutical eqivalence

As defined in the orange book to be pharmaceutically equivalent, the generic and pioneer formulations must (a) contain the same active ingredient (b) contain the same strength of active ingredient in the same dosage form (c) be intended for the same route of administration and (d)be labelled for the same conditions of use.The FDA does not reqire that the generic and reference listed controlled released product contain the same exipients , or that the mechanism by which the release of active drug substance from the formulation be the same. For substitution purposes the two products have to be pharmaceutically equivalent. (Marroum 1997)

Bioeqivalence requirements

The same BE requirements apply to the establishment of the equivalence of the formulation used in efficacy trials if it is different from the formulation intended for marketing and generic controlled-release product approval. For MR products submitted as ANDAs, the following studies are recommended:

1. A single-dose replicate fasting study comparing the highest strength of the test and reference listed drug product.

2. A food effect nonreplicate study comparing the highest strength of the test and reference listed drug product. A typical meal consists of the following: 2 eggs fried in butter,2 strips of bacon,2 slices of toast with butter,4 oz of hash brown potatoes,8 oz of whole milk. Alternatively other meals with 1000 caloric content, with 50% of the calories derived from fat, could be used. The dosage from should be administered immediately following the completion of the breakfast or meal.

Since single-dose studies are considered more sensitive in addressing the primary question of BE(release of the drug at the same rate to the same extent),multiple doses are no longer more recommended even in instance where nonlinear kinetics are present. This is departing from the long-standing policy that was outlined in the 1993 guidance issued by the office of Generic Drugs to also require controlled-release generic products to be bioequivalent under steady-state conditions.

For controlled release formulations marketed in multiple strengths,a single dose BE study under fasting conditions is required only on the highest strengths,provided that the composition of lower strengths are proportional to that of the highest strengths are manufactured under the same conditions.single-dose BE studies may be waived for the lower strengths on the basis of acceptable dissolution profiles.for controlled release products that are not compositionaly proportional,a single dose BE study is required for each strengths.this requirement can also be waived in the presence of an IVIVC whose predictability has been established.for tha waiver to be granted on the basis of an acceptable IVIVC,the following conditions have to be met:

Have the same release mechanism. Have similar in vitro dissolution profiles. Are manufactured using the same type of equipment, the same process, and the same site as other strengths that have bioavailability data. In addition, one of the following situations should exist:

BE has been established for all strengths of the references listed product. Dose proportionalityhas been established for references listed for all strengths product,and all reference product strengths are compositionally proportional,have the same release mechanism,and the invitro dissolution profiles for all strengths are similar. BE is established between the generic product and the references listed product at the highest and the lowest strengths,and for the reference listed product,all strengths are compositionally proportionally or qualitatively the same,have the same release mechanism,and the invitro dissolution profiles are similar.

The criteria for granting such waivers is that difference in predicted means of Cmax and AUC is no more than 10%based on dissolution profiles of the highest strength and the lower strength product.

Scale-Up and Postapproval Changes For Modified Release Product

This guidance provides recommendations to pharmaceutical sponsors of new drug applications (NDAs), abbreviated new drug applications (ANDAs), and abbreviated antibiotic drug applications (AADAs) who intend to change (1) the components or composition, (2) the site of manufacture, (3) the scale-up/scale-down of manufacture, and/or (4) the manufacturing (process and equipment) of a modified release solid oral dosage form during the postapproval period.

FDA regulations at 21 CFR 314.70(a) provide that applicants may make changes to an approved application in accordance with a guidance, notice, or regulation published in the Federal Registerthat provides for a less burdensome notification of the change (for example, by notification at thetime a supplement is submitted or in the next annual report). This guidance permits lessburdensome notice of certain postapproval changes within the meaning of § 314.70(a).For postapproval changes for modified release solid oral dosage forms that affect components andcomposition, scale-up/scale-down, site change, and manufacturing process or equipment changes, this guidance supersedes the recommendations in section 4.G of the Office of Generic Drugs (OGD) Policy and Procedure Guide 22-90 (September 11, 1990). For all other dosage forms andchanges, this guidance does not affect the recommendations in Guide 22-90.

General stability considerations

The effect SUPAC-type changes have on the stability of the drug product should be evaluated.

For general guidance on conducting stability studies, applicants are referred to the FDA Guidelinefor Submitting Documentation for the Stability of Human Drugs and Biologics (02/87). For SUPAC submissions, the following points also should be considered:

In most cases (except those involving scale up), stability data from pilot scale batches will be acceptable to support the proposed change.

Where stability data show a trend toward potency loss or degradant increase under accelerated conditions, it is recommended that historical accelerated stability data from a representative prechange batch be submitted for comparison. It is also recommended that under these circumstances, all available long-term data on test batches from ongoing studies be provided in the supplement. Submission of historical accelerated and available long-term data would facilitate review and approval of the supplement.

A commitment should be included to conduct long-term stability studies through the expiration dating period, according to the approved protocol, on the first or first three (see text for details) production batches and to report the results in the annual reports.

Components and composition — non release controlling

Excipient

This section of the guidance focuses on changes in nonrelease controlling excipients in the drug product. For modified release solid oral dosage forms, consideration should be given as to whether the excipient is critical or not critical to drug release. The sponsor should provide appropriate justifications for claiming any excipient(s) as a nonrelease controlling excipient in the formulation of the modified release solid oral dosage form. The functionality of each excipient 3 should be identified. Changes in the amount of the drug substance are not addressed by this guidance. Changes in components or composition that have the effect of adding a new excipient or deleting an excipient are defined at level 3 (defined below), except as described below in Section III.A.1.a. Waiver of bioequivalence testing for a change in composition which involves only a different color, flavor or preservative may be permissible as described in 21 CFR 320.22(d)(4).

A. Level 1 Change

1. Definition of Level

Level 1 changes are those that are unlikely to have any detectable impact on formulation quality and performance.

Examples:

a. Deletion or partial deletion of an ingredient intended to affect the color or flavor of the drug product; or change in the ingredient of the printing ink to another approved ingredient.

b. Changes in nonrelease controlling excipients, expressed a percentage (w/w) of total formulation, less than or equal to the following percent ranges:

Nonrelease Controlling Excipient	**Percent Excipient (w/w) Out Of Total Target Dosage Form weight**
Filler	±5
Disintegrant	
Starch	±3
Other	±1
Binder	±0.5
Lubricant	
Ca or Mg Stearate	±0.25
Other	±1
Glidant	
Talc	±1
Other	±0.1
Film Coat	±1

These percentages are based on the assumption that the drug substance in the product is formulated to 100% of label/potency. The total additive effect of all non release controlling excipient changes should not be more than 5%. The total weight of the dosage form should still be within the original approved applicationrange. The components (active and excipients) in the formulation should have numerical targets that represent the nominal composition of the drug product on which any future changes in the composition of the product are to be based. Allowable changes in the composition should be based on the original approved target composition and not on previous level 1 changes in the composition. For products approved with only a range for excipients, the target value may be assumed to be the midpoint of the original approved application range.

2. Test Documentation

a. Chemistry documentation
Application/compendial product release requirements. Stability: First production batch on long-term stability data reported in annual report.

b. Dissolution documentation
None beyond application/compendial requirements.

c. Bioequivalence documentation
None.

3. Filing Documentation
Annual report (all information including long-term stability data).

B. Level 2 Change

1. Definition of Level

Level 2 changes are those that could have a significant impact on formulation quality and performance.

Examples:

a. A change in the technical grade and/or specifications of a non-release controlling excipient.3

b. Changes in nonrelease controlling excipients, expressed as percentage (w/w) of total formulation, greater than those listed above for a level 1 change, but less than or equal to the following percent ranges (which represent a two-fold increase over level 1changes):

Non release controlling excipient	Percent excipient (W/W) out of total target dosage form weight
Filler	±10
Disintegrant Starch other	 ±6 ±2
binder	±1
Lubricant Ca or Mg Stearate other	 ±5 ±2
Glidant Talc other	 ±2 ±0.2
Film coat	±2

These percentages are based on the assumption that the drug substance in the drug product is formulated to 100% of label/potency. The total additive effect of all nonrelease controlling excipient changes should not change by more than 10%. The total weight of the dosage form could still be within or outside the original approved application range. The components (active and excipients) in the formulation should have numerical targets that represent the nominal composition of the product on which any future changes in the composition of the product are to be based. Allowable changes in the composition are based on the original approved target composition and not on the composition based on previous level 1 or level 2 changes. For products approved with only a range for excipients, the target value may be assumed to be the midpoint of the original approved application range.

2. Test documentation

a. Chemistry documentation

Application/compendial product release requirements and updated executed batch records. Stability: One batch with three months accelerated stability data reported in prior approval supplement and long-term stability data of first production batch reported in annual report.

b. Dissolution documentation

Extended release: In addition to application/compendial release requirements, multipoint dissolution profiles should be obtained in three other media, for example, in water, 0.1N HCl, and USP buffer media at pH 4.5, and 6.8 for the changed drug product and the biobatch or marketed batch (unchanged drug product). Adequate sampling should be performed, for example, at 1, 2, and 4 hours and every two hours thereafter until either 80% of the drug from the drug product is released or an asymptote is reached. A surfactant may be used with appropriate justification.

Delayed release: In addition to application/compendial release requirements, dissolution tests should be performed in 0.1 N HCl for 2 hours (acid stage) followed by testing in USP buffer media, in the range of pH 4.5-7.5 (buffer stage) under standard (application/compendial) test conditions and two additional agitation speeds using the application/ compendial test apparatus (three additional test conditions). If the application/compendial test apparatus is the rotating basket method (Apparatus 1), a rotation speed of 50, 100, and 150 rpm may be used, and if the application/compendial test apparatus is the rotating paddle method (Apparatus 2), a rotation speed of 50, 75, and 100 rpm may be used. Multipoint dissolution profiles should be obtained during the buffer stage of testing. Adequate sampling should be performed, for example, at 15, 30, 45, 60, and 120 minutes (following the time from which the dosage form is placed in the buffer) until either 80% of the drug from the drug product is released or an asymptote is reached. The above dissolution testing should be performed using the changed drug product and the biobatch or marketed batch (unchanged drug product).

All modified release solid oral dosage forms: In the presence of an established in vitro/in vivo correlation

(6), only application/compendia dissolution testing need be performed (i.e., only in vitro release data by the correlating method need to be submitted). The dissolution profiles of the changed drug product and the biobatch or marketed batch (unchanged drug product) should be similar. The sponsor should apply appropriate statistical testing with justifications (e.g., the f equation) for comparing 2 dissolution profiles

(5). Similarity testing for the two dissolution profiles (i.e., for the unchanged drug product and the changed drug product) obtained in each individual medium is appropriate.

c. Bioequivalence documentation
None.

3. Filing Documentation
Prior approval supplement (all information including accelerated stability data); annual report (long-term stability data).

C. Level 3 Change

1. Definition of Level

Level 3 changes are those that are likely to have a significant impact on formulation quality and performance.

Example:

a. Changes in the nonrelease controlling excipient range beyond those listed in Section III.B.1.

b. The total weight of the dosage form may be within or outside the approved original application range.

2. Test Documentation

a. Chemistry documentation

Application/compendial product release requirements and updated executed batch records.

Stability:

Significant body of information available: One batch with three months' accelerated stability data reported in prior approval supplement and longterm stability data of first three production batches reported in annual report.

Significant body of information not available: Three batches with three months' accelerated stability data reported in prior approval supplement and long-term stability data of first three production batches reported in annual report

b. Dissolution documentation

Extended release: In addition to application/compendial release requirements, a multipoint dissolution profile should be obtained using the application/compendial test conditions for the changed drug product and the biobatch or marketed batch (unchanged drug product). Adequate sampling should be performed, for example, at 1, 2, and 4 hours and every two hours thereafter, until either 80% of the drug from the drug product is released or an asymptote is reached.

Delayed release: In addition to application/compendial release requirements, a multipoint dissolution profile should be obtained during the buffer stage of testing using the application/compendial test conditions for the changed drug product and the biobatch or marketed batch (unchanged drug product). Adequate sampling should be performed, for example at 15, 30, 45, 60, and 120 minutes (following the time from which the dosage form is placed in the buffer) until either 80% of the drug from the drug product is released or an asymptote is reached.

c. Bioequivalence documentation

A single-dose bioequivalence study.The bioequivalence study may be waived in the presence of an established in vitro/in vivo correlation.

3. Filing Documentation

Prior approval supplement (all information including accelerated stability data); annual report (long-term stability data).

IV. Components and composition — release controlling

Excipient

This section of the guidance focuses on changes in release controlling excipients in the drug product. For modified release solid oral dosage forms, consideration should be given as to whether or not the excipient is critical to drug release. The sponsor should provide appropriate justifications (i.e., mechanism of drug release and manufacturing process) for claiming any excipient(s) as a release controlling excipient in the formulation of the modified release solid oraldosage form. The functionality of each excipient should be identified. Changes in the amount of the drug substance are not addressed by this guidance. Changes exceeding the ranges defined in each of the levels below may be allowed if considered to be within normal batch-to-batch variation and contained within an approved original application. In such situations, sponsors should contact the appropriate CDER review division for further guidance. (Marroum 1997)

A. Level 1 Change

1. Definition of Level

Level 1 change is that which are unlikely to have any detectable impact on formulation quality and performance.

Example:

a. Changes in the release controlling excipient(s), expressed as percentage (w/w) of total release controlling excipient(s) in the formulation less than or equal to 5% w/w of total release controlling excipient content in the modified release solid oral dosage form.

The drug substance in the product is formulated to 100% of label/potency. The total additive effect of all release controlling excipient changes should not be more than 5% w/w of the total release controlling excipients in the original approved formulation.4 The total weight of the dosage form should still be within the approved original application range.

The components (active and excipients) in the formulation should have numerical targets that represent the nominal composition of the product on which any future changes in the composition of the product are to be based. Allowable changes in the composition should be based on the original approved target composition and not on previous level 1 changes in the composition. For products approved with only a range for excipients, the target value may be assumed to be the midpoint of the original approved application range.

2. Test Documentation

a. Chemistry documentation

Application/compendial product release requirements.

Stability: First production batch on long-term stability data reported in annual report.

b. Dissolution documentation

None beyond application/compendial requirements.

c. Bioequivalence documentation
None.

3. Filing Documentation
Annual report (all information including long-term stability data). (Marroum 1997)

B. Level 2 Change

1. Definition of Level

Level 2 changes are those that could have a significant impact on formulation quality and performance. Test documentation for a level 2 change would vary depending on whether the product could be considered to have a narrow therapeutic range.
Examples:

a. Change in the technical grade and/or specifications of the release controlling excipient(s).

b. Changes in the release controlling excipient(s), expressed aspercentage (w/w) of total release controlling excipient(s) in the formulation, greater than those listed above for a level 1 change, but less than or equal to 10% w/w of total release controlling excipient content in the modified release solid oral dosage form.
The drug substance in the drug product is formulated to 100% of label/potency. The total additive effect of all release controlling excipient changes should not be more than 10% w/w of the total release controlling Excipient in the original approved formulation. The total weight of the dosage form could still be within or outside the approved original application range.

The components (active and excipients) in the formulation should have numerical targets that represent the nominal composition of the product on which any future changes in the composition of the product are to be based. Allowable changes in the composition are based on the original approved target composition and not on the composition based on previous level 1 or level 2 changes. For products approved with only a range for excipients, the target value may be assumed to be the midpoint of the original approved application range.

2. Test Documentation

a. Chemistry documentation

Application/compendial product release requirements and updated executed batch records.

Stability:

Nonnarrow therapeutic range drugs: One batch with three months' accelerated stability data reported in prior approval supplement and long-term stability data of first production batch reported in annual report.

Narrow therapeutic range drugs: Three batches with three months' accelerated stability data reported in prior approval supplement and long-term stability data of first three production batches reported in annual report.

b. Dissolution documentation
Non-narrow therapeutic range drugs

Extended release: In addition to application/compendial release requirements, multipoint dissolution profiles should be obtained in three other media, for example, in water, 0.1N HCl, and USP buffer media at pH 4.5, and 6.8 for the changed drug product and the biobatch or marketed batch (unchanged drug product). Adequate sampling should be performed, for example, at 1, 2, and 4 hours and every two hours thereafter until either 80% of the drug from the drug product is released or an asymptote is reached. A surfactant may be used with appropriate justification.

Delayed release: In addition to application/compendial release requirements, dissolution tests should be performed in 0.1 N HCl for 2 hours (acid stage) followed by testing in USP buffer media in the range of pH 4.5-7.5 (buffer stage) under standard (application/compendial) test conditions and two additional agitation speeds using the application/compendial test apparatus (three additional test conditions). If the application/compendial test apparatus is the rotating basket method (Apparatus 1), a rotation speed of 50, 100, and 150 rpm may be used, and if the application/compendial test apparatus is the rotating paddle method (Apparatus 2), a rotation speed of 50, 75, and 100 rpm may be used. Multipoint dissolution profiles should be obtained during the buffer stage of testing. Adequate sampling should be performed, for example, at 15, 30, 45, 60, and 120 minutes (following the time from which the dosage form is placed in the buffer) until either 80% of the drug from the drug product is released or an asymptote is reached. The above dissolution testing should be performed using the changed drug product and the biobatch or marketed batch (unchanged drug product). All modified release solid oral dosage forms: In the presence of an established in vitro/in vivo correlation (6), only application/compendia dissolution testing should be performed (i.e., only in vitro release data by the correlating method should be submitted). The dissolution profiles of the changed drug product and the biobatch or marketed batch (unchanged drug product) should be similar. The sponsor should apply appropriate statistical testing with justifications (e.g., the f equation) for comparingdissolution profiles (5). Similarity testing for the two dissolution profiles (i.e., for the unchanged drug product and the changed drug product) obtained in each individual medium is appropriate.

Narrow therapeutic range drugs

Extended release: In addition to application/compendial release requirements, a multipoint dissolution profile should be obtained in application/compendial medium for the changed drug product and the biobatch or marketed batch (unchanged drug product). Adequate sampling should be performed, for example at 1, 2, and 4 hours and every two hours
There after until either 80% of the drug from the drug product is released or an asymptote is reached.

Delayed release: In addition to application/compendial release requirements, a multipoint dissolution profile should be obtained during the buffer stage of testing using the application/compendial medium for the changed drug product and the biobatch or marketed batch (unchanged drug product). Adequate sampling should be performed, for example, at 15, 30, 45, 60, and 120 minutes (following the time from which the dosage form is placed in the buffer) until either 80% of the drug from the drug product is released or an asymptote is reached.

c. Bioequivalence documentation
Non-narrow therapeutic range drugs: None.
Narrow therapeutic range drugs: A single-dose bioequivalence study. The bioequivalence study may be waived in the presence of an established in vitro/in vivo correlation. Changes in release controlling excipients in the formulation should be within the range of release controlling excipients of the established correlation.

3. Filing Documentation
Prior approval supplement (all information including accelerated stability data); annual report (long-term stability data). (Marroum 1997)

C. Level 3 Change

1. Definition of Level

Level 3 changes are those that are likely to have a significant impact on formulation quality and performance affecting all therapeutic ranges of the drug.
Examples:

a. Addition or deletion of release controlling excipient(s) (e.g., release controlling polymer/plasticizer).

b. Changes in the release controlling excipient(s), expressed as percentage (w/w) of total release controlling excipient(s) in the formulation, greater than those listed above for a level 2 change (i.e., greater than 10% w/w of total release controlling excipient content in the modified release solid oral dosage form). Total weight of the dosage form may be within or outside the original approved application range.

2. Test Documentation

a. Chemistry documentation

Application/compendial product release requirements and updated executed batch records.

Stability: Three batches with three months' accelerated stability data reported in prior approval supplement and long-term stability data of first three production batches reported in annual report.

b. Dissolution documentation

Extended release: In addition to application/compendial release requirements, a multipoint dissolution profile should be obtained using application/compendial test conditions for the changed drug product and the biobatch or marketed batch (unchanged drug product). Adequate sampling should be performed, for example at 1, 2, and 4 hours and every two hours thereafter until either 80% of the drug from the drug product is released or an asymptote is reached.

Delayed release: In addition to application/compendial release requirements, a multipoint dissolution profile should be obtained during the buffer stage of testing using the application/compendial test conditions for the changed drug product and the biobatch or marketed batch (unchanged drug product). Adequate sampling should be performed, for example at 15, 30, 45, 60, and 120 minutes (following the time from which the dosage form is placed in the buffer) until either 80% of the drug from the drug product is released or an asymptote is reached.

c. Bioequivalence documentation

A single-dose bioequivalence study (3). The bioequivalence study may be waived in the presence of an established in vitro/in vivo correlation (6). Changes in release controlling excipients in the formulation should be within the range of release controlling excipients of the established correlation.

3. Filing Documentation
Prior approval supplement (all information including accelerated stability data); annual report (long-term stability data).
(Marroum 1997)

Table.1: Summary of Requirments For level of Changes In Equipment For Extened Release Dosage Forms.

Level	Classification	Changes	Test documentation	Filing documentation
I	Equipment changes No other changes (All drug)	Alternate equipment of same design and principle Automated equipment	Updated batch record Stability Application/compendial requirements No biostudy	Annual report
II	Equipment changes No other changes (All drug)	Change to equipment of different design and operating principle	Updated batch record Stability Application/compendial requirements plus multi-point dissolution profiles in three other media (e.g., water, 0.1N HCL, and USP buffer media at pH 4.5 and 6.8)until 80% of drug released or an asymptote is reached Apply some statistical test (F2 test)for compairing dissolution profilesb No biostudy	Prior approval supplement

Table.2: Summary of Requirments For level of Changes in Manufacturing Site for Extened Release Dosage Forms.

Level	Classification	Therapeutic range	Test documentation	Filing documentation
I	Single facility Common personnel No other changes	All drug	Application/compendial requirements No biostudy	Annual report
II	Same contiguous campus Common personnel No other changes	All drug	Identification and description of site of change, and updated batch record Notification of site change Stability Application/compendial requirements plus multi-point dissolution profiles in three other media (e.g., water, 0.1N HCL, and USP buffer media at pH 4.5 and 6.8)until 80% of drug released or an asymptote is reached Apply some statistical test (F2 test)for compairing dissolution profilesb No biostudy	Changes being effected supplement
III	Different campus Different personnel	All drug	Notification of site change Updated batch recored Stability Application/compendail(profile)requirements Biostudy or IVIVC	Prior approval supplement

Table.3: Summary of Requirments For level of Changes In Manufacturing Process For Extened Release Dosage Forms.

Level	Classification	change	Test documentation	Filing documentation
I	Process changes affecting the non-release controlling excipients and/or the release controlling excipients No other changes	Adjustment of equipment operating condition (e.g. mixing time, operating speed, etc.) Within the approved application ranges	Updated batch recored Application/compendail(profile)requirements No biostudy	Annual Report
II	Processing changes affecting the non-release controlling excipients and/or the release controlling excipients No other changes	Adjustment of equipment operating condition (e.g. mixing time, operating speed, etc.) Beyond approved application ranges	Updated batch recored Stability Application/compendia requirements plus multi-point dissolution profiles in three other media (e.g., water, 0.1N HCL, and USP buffer media at pH 4.5 and 6.8)until 80% of drug released or an asymptote is reached Apply some statistical test (F2 test) for comparing dissolution profiles No biostudy	Changes being effected suppement
III	Processing changes affecting the non-release controlling excipients and/or the release controlling excipients	Changes in the type of process used (e.g., from wet granulation to direct compression)	Updated batch recored Stability Application/compendail(profile)requirements Biostudy or IVIVC	Prior approval supplement

Table.4: Summary of Requirments For level of Changes in Scale-up/Scale-down For Extened Release Dosage Forms.

Level	Classification	change	Test documentation	Filing documentation
I	Scale-up of bio-batch(s)or pivotal clinical batch(s)s No other change	-10X (All drugs	Updated batch recored Stability Application/compendail(profile)requirements No biostudy	Annual Report
II	Scale-up of bio-batch(s)or pivotal clinical batch(s)s No other change	>10X (All drugs	Updated batch recored Stability Application/compendial requirements plus multi-point dissolution profiles in three other media (e.g., water, 0.1N HCL, and USP buffer media at pH 4.5 and 6.8)until	Changes being effected suppement

			80% of drug released or an asymptote is reached Apply some statistical test (F2 test) for comparing dissolution profiles No biostudy	

Table.5: Summary of Requirments For level of Changes in Scale-up/Scale-down For Delayed Release Dosage Forms .

Level	Classification	change	Test documentation	Filing documentation
I	Scale-up of bio-batch(s)or pivotal clinical batch(s)s No other change	-10X (All drugs	Updated batch recored Stability Application/compendail(profile)requirements No biostudy	Annual Report
II	Scale-up of bio-batch(s)or pivotal clinical batch(s)s No other change	>10X (All drugs	Updated batch recored Stability Application/compendial requirements plus multi-point dissolution profiles in additional buffer stage testing (e.g., USP buffer media at pH 4.5 and 7.5) under standard and increased agitation conditions until 80% of released or an asymptote is reached Apply some statistical test (F2 test) for comparing dissolution profiles No biostudy	Changes being effected supplement

Table.6: Summary of Requirments For level of Changes in Manufacturing Site For Delayed Release Dosage Forms.

Level	Classification	Therapeutic range	Test documentation	Filing documentation
I	Single facility Common personnel No other changes	All drug	Application/compendial requirements No biostudy	Annual Report
II	Same contiguous campus Common personnel No other changes	All drug	Identification and description of site of change, and updated batch record Notification of site change Stability Application/compendial requirements plus multi-point dissolution profiles in additional buffer stage testing (e.g., USP buffer media at pH 4.5 and 7.5) under standard and increased agitation conditions until 80% of released or an asymptote is reached Apply some statistical test (F2 test) for	Changes being effected supplement

			comparing dissolution profiles No biostudy	
III	Different campus Different personnel	All drug	Notification of site change Updated batch recored Stability Application/compendail (profile)requirements Biostudy or IVIVC	Prior approval supplement

Table.7: Summary of Requirments For level of Changes In Non-Release-Controlling Component and Composition For Delayed Release Dosage Forms.

Level	Classification	Therapeutic range	Test documentation	Filing documentation
I	Complete or partial deletion of color/ flavor Change in inks, imprints Upto SUPAC-IR level 1 excipient ranges No other changes	All drug	Stability Application/compendial requirements No biostudy	Annual Report
II	Changes in technical grade and/or specifications Higher than SUPAC-IR level 1 but less than level 2 excipient ranges No other changes	All drug	Notification and updated batch record Stability Application/compendial requirements plus multi-point dissolution profiles in additional buffer stage testing (e.g., USP buffer media at pH 4.5 and 7.5) under standard and increased agitation conditions until 80% of released or an asymptote is reached Apply some statistical test (F2 test) for comparing dissolution profiles No biostudy	Changes being effected supplement
III	Higher than SUPAC-IR level 2 excipient ranges	All drug	Updated batch record Stability Application/compendail (profile)requirements Biostudy or IVIVC	Prior approval supplement

Table.8: Summary of Requirments For level of Changes in Equipment For Delayed Release Dosage Forms.

Level	Classification	Changes	Test documentation	Filing documentation
I	Equipment changes No other changes (All drug)	Alternate equipment of same design and principle Automated equipment	Updated batch record Stability Application/compendial requirements No biostudy	Annual report

II	Equipment changes No other changes (All drug)	Change to equipment of different design and operating principle	Updated batch record Stability Application/compendial requirements plus multi-point dissolution profiles in additional buffer stage testing (e.g., USP buffer media at pH 4.5 and 7.5) under standard and increased agitation conditions until 80% of released or an asymptote is reached Apply some statistical test (F2 test)for compairing dissolution profiles No biostudy	Prior approval supplement

Table.9: Summary of Requirments For level of Changes In Manufacturing Process For Delayed Release Dosage Forms.

Level	Classification	change	Test documentation	Filing documentation
I	Process changes affecting the non-release controlling excipients and/or the release controlling excipients No other changes	Adjustment of equipment operating condition (e.g. mixing time, operating speed, etc.) Within the approved application ranges	Updated batch recored Application/compendail(profile)requirements No biostudy	Annual Report
II	Processing changes affecting the non-release controlling excipients and/or the release controlling excipients No other changes	Adjustment of equipment operating condition (e.g. mixing time, operating speed, etc.) Beyond approved application ranges	Updated batch record Stability Application/compendial requirements plus multi-point dissolution profiles in additional buffer stage testing (e.g., USP buffer media at pH 4.5 and 7.5) under standard and increased agitation conditions until 80% of released or an asymptote is reached Apply some statistical test (F2 test)for compairing dissolution	Changes being effected suppement

			profiles No biostudy	
III	Processing changes affecting the non-release controlling excipients and/or the release controlling excipients	Changes in the type of process used (e.g., from wet granulation to direct compression)	Updated batch recored Stability Application/compendail(profile)requirements Biostudy or IVIVC	Prior approval supplement

Table.10: Summary of Requirments For level of Changes in Release Controlling-Component and Conposion For Extened Release Dosage Forms.

Level	Classification	Therapeutic range	Test documentation	Filing documentation
I	-5% W/W changes based on total release controlling excipient (e.g., controlled release polymer, plasticizer) content No other changes	All drug	Stability Application/compendail(profile)requirements No biostudy	Annual Report
II	Change in technical grade and/or specifications – 10% W/W change based on total release controlling excipient (e.g., controlled release polymer, plasticizer) content No other changes	Nonnarrow	Notification and updated batch record Stability Application/compendial requirements plus multi-point dissolution profiles in additional buffer stage testing (e.g., USP buffer media at pH 4.5 and 7.5) under standard and increased agitation conditions until 80% of released or an asymptote is reached Apply some statistical test (F2 test)for compairing dissolution profiles No biostudy	Prior approval supplement
		Narrow	Updated batch recored Stability Application/compendail(profile)requirements Biostudy or IVIVC	Prior approval supplement
III	>10%W/W changes based on total release-controlling excipient (e.g., controlled release polymer, plasticizer) content	All drug	Updated batch recored Stability Application/compendail(profile)requirements Biostudy or IVIVC	Prior approval supplement

IN VITRO DISSOLUTION FOR MODIFIED-RELEASE FORMULATIONS

A. IVIVC

Since the release of the drug is the rate-limiting step of the appearance of the drug in the systemic circulation for controlled-release formulation, it is therefore possible to establish a relationship between the in vitro dissolution of the drug and its in vivo performance. A USP PF stimuli article published in 1988 established three levels of correlation:

Level A correlation is defined as appoint-to-point relationship between the in vitro dissolution profile and the in vivo input rate (usually expressed as the fraction of drug absorbed versus time). This type of correlation is considered to be the most useful from a regulatory point of view.

Level B correlation: uses the principles of statistical moment analysis. In such a correlation, for example, the mean in vitro dissolution time is correlated with the mean residence time or the mean absorption time in vivo. Such a correlation suffers from the fact that a number of different in vivo profiles can produce the same mean absorption time in vivo. Thus, Level B correlations are considered of little value from a regulatory point of view.

Level C correlation: establishes a relationship between the amounts of drug dissolved at a certain pharmacokinetic parameter. A multiple level C correlation, on the other hand, relates one or more PK parameter(s) such as AUC or Cmax to the amount of drug dissolved at several time points of the dissolution profile.

In September 1997, the FDA issued guidance on this topic to provide recommendation for the development, evaluation, and application of IVIVCs. (Marroum 1997)

1. Development of IVIVC

Human data should be utilized for regulatory consideration of an IVIVC. Bioavailability studies for IVIVC development should be performed with enough subjects to characterize adequately the performance of the drug product under study. Although crossover studies are preferred, parallel studies or cross-study analyses may be acceptable. The references product in developing an IVIVC may be an intravenous solution, an aqueous oral solution, or an IR product. IVIVCs are usually developed in the fasted state. When a drug is not tolerated in the fasted state, studies may be conducted in the fed state. Any in vitro dissolution method may be used to obtained the dissolution characteristics of the oral controlled release dosage form but the same system should be used for all formulations tested.The preferred dissolution apparatus is USP apparatus 1 (basket) or 2 (paddle), used at compendially recognized rotation speeds (e.g. 100 rpm for the basket and 50-75 rpm for the paddle). In other cases, the dissolution properties of some oral controlled-release formulations may be determined with USP apparatus 3 (reciprocating cylinder) or IV (flow through cell). An aqueous medium, either water or a buffered solution preferably not exceeding pH 6.8, is recommended as the initial medium for development of an IVIVC. For poorly soluble drugs, addition of surfact ant (e.g. 1% sodium lauryl sulphate) may be appropriate.

IVIVCs are established in two stages. First, the relationship between dissolution charactrestics and bioavailability characteristics needs to be determined. Second, the relationship must be tesed. The first stage may be thought of as developing an IVIVC, whereas the second stage may involve evaluation of predictability. The most commonly seen process for developing a level A IVIVC is to:

1. Develop formulation with different release rates, such as slow, medium, fast, or a single release rate if dissolution is condition independent.
2. Obtain in vitro dissolution profiles and in vivo plasma concentration profiles for these formulations.

Estimate the in vivo absorption or dissolution time course using an appropriate deconvolution technique for each formulation and subject (e.g. Wagner-Nelson, numerical deconvulation). These three step establish the IVIVC model. Alternative approchese to developing level A IVIVCs are possible. The IVIVC relationship should be demonstrated consistently with two or more formulations with different release rate to result in corresponding differences in absoption profiles. Exception to this approach (i.e. use of only one formulation) may be considered for formulations for which in vitro dissolution is independent of the dissolution test conditions (e.g. medium, agitation,, ph).

The in vitro dissolution methodology should adequately discriminate among formulations. Dissolution testing can be carried out during the formulation screening stage using several methods. Once the discriminating system is developed, dissolution condition should be same for all formulation tested in the bioavailability study for development of the correlation and should be fixed before further steps toward correlation evaluation are undertaken. During the early stages of correlation development, dissolution conditions may be altered to develop a one-to-one correlation between the in-vitro dissolution profile and the in-vivo dissolution profile.

Time scaling may be used as long as the time scaling factor is the same for all formulations. (Marroum 1997)

Evaluation of an IVIVC

An IVIVC that has been developed should be evaluated to demonstrate that predictability of the in-vivo performance of a drug product from its in-vitro dissolution characteristics is maintained over a range of in-vitro dissolution release rates and manufacturing changes. Since the objective of developing an IVIVC is to establish a predictive mathematical model describing the relationship between an in-vitro property and a relevant in-vivo response, a logical evaluation approach focuses on the estimation of predictive performance or, conversely, prediction error. Depending on the intended application of an IVIVC and the therapeutic index of the drug, evaluation of prediction error internally and/ or externally may be appropriate. Evaluation internal predictability is based on the initial data used to develop the IVIVC model. Evaluation of external predictability is based on additional test data sets.

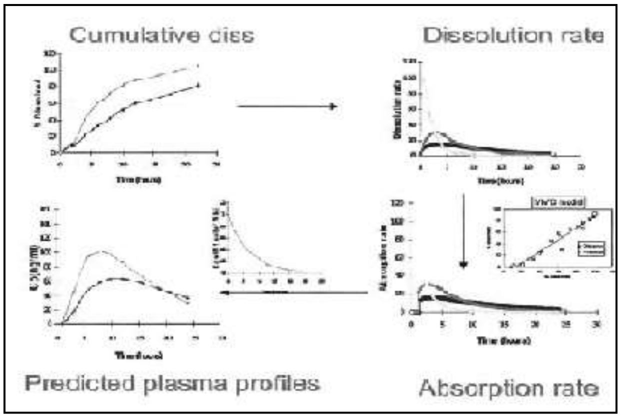

Fig.1: The convolution procedure to predict plasma concentrations from dissolution profiles.

Figure 1 shows the procedure by which one predicts the plasma concentration time profile of an MR formulation from its in-vitro dissolution.

The dissolution profiles are fitted to mathematical function. The cumulative dissolution profiles are converted to dissolution rates by taking the first derivative of this function. The dissolution rate is then converted into an absorption rate by using IVIVC relationship. The predicted plasma concentrations are then obtained by convolving the absorption rates with the disposition function of the drug.

For internal predictability, the IVIVC is deemed acceptable if the average prediction error for all formulations used to develop the correlation for C_{max} and AUC is less than 10% with none exceeding 50%. If the criteria for internal predictability are not met, then one would proceed to evaluation of external predictability. If the prediction errors are less than 10%, the IVIVC is deemed acceptable, if the prediction errors are more than 15% on average, the IVIVC is deemed inconclusive and further evaluation would be needed. This can be accomplished by attempting to predict additional data sets. If the prediction errors are on an average above 20%, the IVIVC is considered to be of poor predictive ability and is considered unacceptable from a regulatory point of view. (Marroum 1997)

3) Applications of IVIVCs:

Once a predictive IVIVC has been developed, in-vitro dissolution cannot only serve as an important tool for (a) providing process control and quality assurance and (b) determining stable release characteristic over time and facilitating the effect of minor formulation changes on the release characteristics of the drug product, but can also serve as a surrogate for the in-vivo performance of the product. This will in turn reduce the regulatory burden on the industry by reducing the number of studies required for the approval and maintenance on the market of a controlled-release product.

With an IVIVC, waivers for more significant changes are possible. A biowaiver will likely be granted for an oral controlled release drug product using an IVIVC for:

1. Level 3 process changes as defined in SUPAC-MR.
2. Complete removal of, or replacement of, non-release controlling excipients as defined in SUPAC-MR.
3. Level 3 changes in release-controlling excipients as defined in SUPAC-MR.
4. Level 3 site change.

The criteria for granting an in-vivo BA/BE waiver are that the predicted mean C_{max} and AUC for the test and reference formulation should defer by no more than 20% as illustrated in figure 2.

If an IVIVC is developed with the highest strength, waivers for changes made on the highest strength and any lower strength may be granted if these strengths are compositionally proportional or qualitatively the same, the in-vitro dissolution profiles of all the strengths are similar and all the strengths have the same release mechanism. (Marroum 1997)

This biowaiver is applicable to the strengths lower than the highest strength, within the dosing range that has been established to be safe and effective, provided the new strengths are compositionally proportional or qualitatively the same, have the same release mechanism, have similar in-vitro dissolution profiles, and are manufactured using the same type of equipment, and the same process at the same site as other strengths that have BA data available.

Certain changes generally always necessitate in-vivo BA testing and in some cases might necessitate clinical trials even in the presence of an IVIVC.

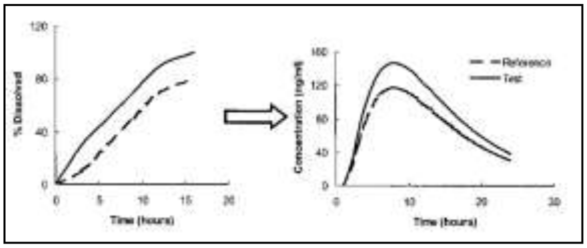

Fig.2: Criteria for granting biowaivers baseon a level A IVIV

These include the approval of a new formulation of an approved oral controlled-release drug product when the new formulation has a different release mechanism, approval of the dosage strength higher or lower than doses that have been shown to be safe and effective in clinical trials, approval of another sponser's oral controlled-release product even with same release controlling mechanism, and approval of a formulation change involving a non-release controlling excipient in the drug product that may significantly affect drug absorption.

B. Setting dissolution specification:

1. No IVIVC present

The dissolution test is an important tool from a quality control point of view. In the past the dissolution specifications were set based on the performance on the clinical/ bio lots. Therefore, if the release characteristics of the formulation were variable and not well controlled, than one would end up with dissolution specifications that are somewhat wider than a formulation with good release characteristics. The end result of this practice was possibility of the introduction on the market of potentially highly variable formulations with different in-vivo performance. This might result in widely fluctuating plasma concentration profiles leading to a variable therapeutic effect and increased incidence of adverse effects and therapeutic failures. The FDA guidance attempted to change this less than optimal practice. The FDA guidance stipulated that the maximum allowable width of a dissolution specifications be no more than 20%± 10% deviation from the dissolution profile of the desired target formulation (see figure 3).

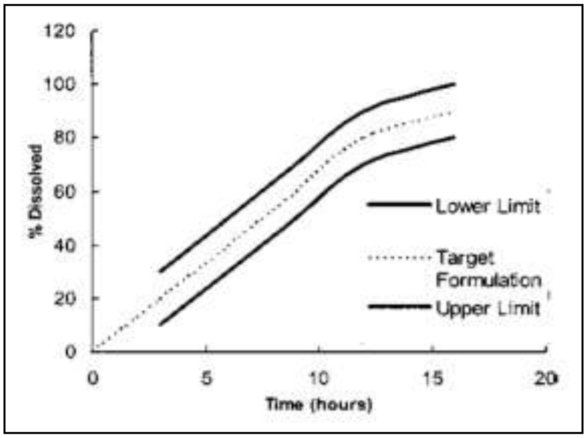

Fig: 3Dissolution specification in the absence of IVIVC.

The guidance recommends that the dissolution specifications include at least three time points, one covering the initial part of the profile, the second on the middle part, and the last one the time when 80% dissolution has occurred or the plateau has been reached if one is unable to obtain complete dissolution. Specifications should be said to pass USP stage II of testing (based on average data). However, if dissolution specification wider than 20% is desired, than one would have to conduct a busy study to show that lots at the upper and lower limit of the specification are bioequivalent. (Marroum 1997)

2. Level A IVIVC Present

In the presence of an IVIVC, the criteria are shifted from the invitro side to the invivo side. In this case, the IVIVC is used to predict the plasma concentration time profile that corresponds to lots that are on the upper and lower limit of the dissolution specification.

Acceptable dissolution specification limits are limits and that do not result in plasma concentration time profiles that differ by more than 20% in the area under the plasma concentration time(AUC) and peak plasma concentration(C_{max})(usually ± 10% of a target clinical/bio formulation). Therefore, it is possible to obtain dissolution specification limits that are wider than 20% if the predicted limits do not result in plasma concentration time profiles that are different in their mean predicted C_{max} or AUC by more than 20%. However, the guidance does not penalize sponsors if the 20%width in the limits results in more than 20% difference in the predicted pharmacokinetic parameters. Tighter limits than the case where no IVIVC is present will be required only if plasma concentration ranges are needed to avoid potential serious toxicities.

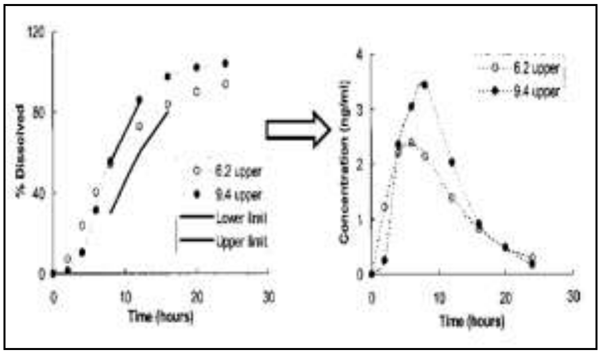

Fig: 4 Influence of the release rate specification on plasma levels: in equivalent plasma profile

With a multiple C correlation, the relationship between the amount of a drug dissolved at each time point and the relevant PK parameters such as C_{max} and AUC should be used to set the dissolution specification limits in such a way that the upper and lower limits do not result in the release of batches that differ by more than 20% in their plasma concentration time profile.

3. Release Rate Specifications

The FDA guidance also allows for a novel approach in setting dissolution specification for formulations exhibiting a zero-order release characteristic.

An example of such a formulation is the osmotic delivery system commonly referred to as gastrointestinal therapeutic system (GITS). If this formulation are designed to deliver the drug at a constant rate that can be described by a linear relationship over a certain period of time, than one can set a release rate specification to describe the performance of the formulation.

This release rate specification can be in addition to, or instead of, the cumulative dissolution specifications that one usually sets for a modified release product.

Having a release rate specification will provide for better control of the invivo performance of the drug because it is the release characteristic of the formulation that will determine the rate of appearance in the systemic circulation. This can be described more appropriately by the relase rate compared to the cumulative amount of drug dissolved at a certain period of time.

As an illustration of this point, consider the dissolution profiles of two lots of the same formulation (show in fig. 4) with similar release rates but which are on the upper and lower limits of the cumulative dissolution specifications. Assuming a level A correlation for this product, the predicted plasma concentration time profile corresponding to these two lots are similar, differing only in the time to achieve peak plasma concentration.

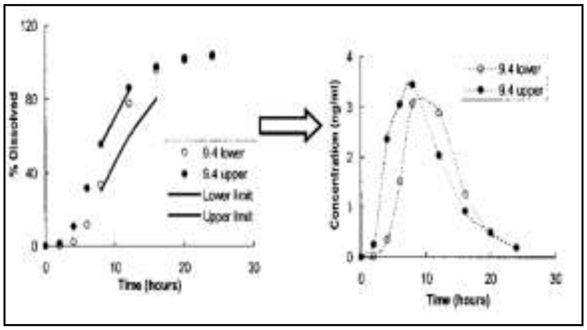

Fig: 5. Influence of the release rate specification on plasma levels: equivalent plasma profile

On the other hand, if one examines the case presented in figure 5, whereby the two lots are very close in their cumulative dissolution profiles (both the upper limit of the dissolution specifications) but are markedly different in their release rates, one can clearly see that the predicted plasma profiles corresponding to these lots are very different ant considered not to be bioequivalent. (Marroum 1997)

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